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Process for the production of polyunsaturated fatty acids in transgenic organisms

The present invention relates to a process for the production of polyunsaturated fatty acids in an organism by introducing, into the organism, nucleic acids which encode polypeptides with Δ5-elongase activity. These nucleic acid sequences, if appropriate together with further nucleic acid sequences which encode polypeptides of the biosynthesis of the fatty acid or lipid metabolism, can advantageously be expressed in the organism. Especially advantageous are nucleic acid sequences which encode a Δ6-desaturase, a Δ5-desaturase, Δ4-desaturase, Δ12-desaturase and/or Δ6-elongase activity. These desaturases and elongases are advantageously derived from Thalassiosira, Euglena or Ostreococcus. The invention furthermore relates to a process for the production of oils and/or triacylglycerides with an elevated content of long-chain polyunsaturated fatty acids.

In a preferred embodiment, the present invention furthermore relates to a method for the production of unsaturated ω3-fatty acids and to a method for the production of triglycerides with an elevated content of unsaturated fatty acids, especially ω3-fatty acids with more than three double bonds. The invention relates to the generation of a transgenic organism, preferably a transgenic plant or a transgenic microorganism, with an elevated content of unsaturated ω3-fatty acids, oils or lipids with ω3-double bonds as the result of the expression of the elongases and desaturases used in the method according to the invention, advantageously in conjunction with ω3-desaturases, for example an ω3-desaturase from fungi of the family Pythiaceae such as the genus Phytophthora, for example the genus and species Phytophthora infestans, or an ω3-desaturase from algae such as the family of the Prasinophyceae, for example the genus Ostreococcus, specifically the genus and species Ostreococcus tauri, or diatoms such as the genus Thalassiosira, specifically the genus and species Thalassiosira pseudonana.

The invention furthermore relates to the nucleic acid sequences, nucleic acid constructs, vectors and organisms comprising the nucleic acid sequences according to the invention, vectors comprising the nucleic acid sequences and/or the nucleic acid constructs and to transgenic organisms comprising the abovementioned nucleic acid sequences, nucleic acid constructs and/or vectors.

A further part of the invention relates to oils, lipids and/or fatty acids produced by the process according to the invention and to their use. Moreover, the invention relates to unsaturated fatty acids and to triglycerides with an elevated content of unsaturated fatty acids and to their use.

Fatty acids and triacylglycerides have a multiplicity of applications in the food industry, in animal nutrition, in cosmetics and in the pharmacological sector. Depending on whether they are free saturated or unsaturated fatty acids or else triacylglycerides with an elevated content of saturated or unsaturated fatty acids, they are suitable for very different applications. Polyunsaturated fatty acids such as linoleic acid and linolenic

acid are essential for mammals, since they cannot be produced by the latter. Polyunsaturated ω3-fatty acids and ω6-fatty acids are therefore an important constituent in animal and human nutrition.

Polyunsaturated long-chain ω3-fatty acids such as eicosapentaenoic acid (= EPA, $C20:5^{\Delta 5,8,11,14,17}$) or docosahexaenoic acid (= DHA, $C22:6^{\Delta 4,7,10,13,16,19}$) are important components in human nutrition owing to their various roles in health aspects, including the development of the child brain, the functionality of the eyes, the synthesis of hormones and other signal substances, and the prevention of cardiovascular disorders, cancer and diabetes (Poulos, A Lipids 30:1-14, 1995; Horrocks, LA and Yeo YK Pharmacol Res 40:211-225, 1999). This is why there is a demand for the production of polyunsaturated long-chain fatty acids.

Owing to the present-day composition of human food, an addition of polyunsaturated ω3-fatty acids, which are preferentially found in fish oils, to the food is particularly important. Thus, for example, polyunsaturated fatty acids such as docosahexaenoic acid (= DHA, $C22:6^{\Delta 4,7,10,13,16,19}$) or eicosapentaenoic acid (= EPA, $C20:5^{\Delta 5,8,11,14,17}$) are added to infant formula to improve the nutritional value. The unsaturated fatty acid DHA is said to have a positive effect on the development and maintenance of brain functions.

Hereinbelow, polyunsaturated fatty acids are referred to as PUFA, PUFAs, LCPUFA or LCPUFAs (<u>p</u>oly <u>u</u>nsaturated <u>f</u>atty <u>a</u>cids, **<u>PUFA</u>**, <u>l</u>ong <u>c</u>hain <u>p</u>oly <u>u</u>nsaturated <u>f</u>atty <u>a</u>cids, **<u>LCPUFA</u>**).

The various fatty acids and triglycerides are mainly obtained from microorganisms such as Mortierella and Schizochytrium or from oil-producing plants such as soybean, oilseed rape, algae such as Crypthecodinium or Phaeodactylum and others, where they are obtained, as a rule, in the form of their triacylglycerides (= triglycerides = triglycerols). However, they can also be obtained from animals, such as, for example, fish. The free fatty acids are advantageously prepared by hydrolysis. Very long-chain polyunsaturated fatty acids such as DHA, EPA, arachidonic acid (= ARA, $C20:4^{\Delta 5,8,11,14}$), dihomo-γ-linolenic acid ($C20:3^{\Delta 8,11,14}$) or docosapentaenoic acid (DPA, $C22:5^{\Delta 7,10,13,16,19}$) are not synthesized in oil crops such as oilseed rape, soybean, sunflower or safflower. Conventional natural sources of these fatty acids are fish such as herring, salmon, sardine, redfish, eel, carp, trout, halibut, mackerel, zander or tuna, or algae.

Depending on the intended use, oils with saturated or unsaturated fatty acids are preferred. In human nutrition, for example, lipids with unsaturated fatty acids, specifically polyunsaturated fatty acids, are preferred. The polyunsaturated ω3-fatty acids are said to have a positive effect on the cholesterol level in the blood and thus on the possibility of preventing heart disease. The risk of heart disease, stroke or hypertension can be reduced markedly by adding these ω3-fatty acids to the food. Also, ω3-fatty acids have a positive effect on inflammatory, specifically on chronically

inflammatory, processes in association with immunological diseases such as rheumatoid arthritis. They are therefore added to foodstuffs, specifically to dietetic foodstuffs, or are employed in medicaments. ω6-Fatty acids such as arachidonic acid tend to have a negative effect on these disorders in connection with these rheumatic diseases on account of our usual dietary intake.

ω3- and ω6-fatty acids are precursors of tissue hormones, known as eicosanoids, such as the prostaglandins, which are derived from dihomo-γ-linolenic acid, arachidonic acid and eicosapentaenoic acid, and of the thromboxanes and leukotrienes, which are derived from arachidonic acid and eicosapentaenoic acid. Eicosanoids (known as the PG_2 series) which are formed from ω6-fatty acids generally promote inflammatory reactions, while eicosanoids (known as the PG_3 series) from ω3-fatty acids have little or no proinflammatory effect.

Owing to the positive characteristics of the polyunsaturated fatty acids, there has been no lack of attempts in the past to make available genes which are involved in the synthesis of these fatty acids or triglycerides for the production of oils in various organisms with a modified content of unsaturated fatty acids. Thus, WO 91/13972 and its US equivalent describes a Δ9–desaturase. WO 93/11245 claims a Δ15-desaturase and WO 94/11516 a Δ12-desaturase. Further desaturases are described, for example, in EP–A–0 550 162, WO 94/18337, WO 97/30582, WO 97/21340, WO 95/18222, EP-A–0 794 250, Stukey et al., J. Biol. Chem., 265, 1990: 20144–20149, Wada et al., Nature 347, 1990: 200–203 or Huang et al., Lipids 34, 1999: 649–659. However, the biochemical characterization of the various desaturases has been insufficient to date since the enzymes, being membrane-bound proteins, present great difficulty in their isolation and characterization (McKeon et al., Methods in Enzymol. 71, 1981: 12141-12147, Wang et al., Plant Physiol. Biochem., 26, 1988: 777–792). As a rule, membrane-bound desaturases are characterized by being introduced into a suitable organism which is subsequently analyzed for enzyme activity by analyzing the starting materials and the products. Δ6–Desaturases are described in WO 93/06712, US 5,614,393, US5614393, WO 96/21022, WO 00/21557 and WO 99/27111 and the application for the production of fatty acids in transgenic organisms is described in WO 98/46763, WO 98/46764 and WO 98/46765. In this context, the expression of various desaturases and the formation of polyunsaturated fatty acids is also described and claimed in WO 99/64616 or WO 98/46776. As regards the expression efficacy of desaturases and its effect on the formation of polyunsaturated fatty acids, it must be noted that the expression of a single desaturase as described to date has only resulted in low contents of unsaturated fatty acids/lipids such as, for example, γ-linolenic acid and stearidonic acid. Moreover, a mixture of ω3- and ω6-fatty acids was obtained, as a rule.

Especially suitable microorganisms for the production of PUFAs are microalgae such as Phaeodactylum tricornutum, Porphiridium species, Thraustochytrium species, Schizochytrium species or Crypthecodinium species, ciliates such as Stylonychia or Colpidium, fungae such as Mortierella, Entomophthora or Mucor and/or mosses such

as Physcomitrella, Ceratodon and Marchantia (R. Vazhappilly & F. Chen (1998) Botanica Marina 41: 553-558; K. Totani & K. Oba (1987) Lipids 22: 1060-1062; M. Akimoto et al. (1998) Appl. Biochemistry and Biotechnology 73: 269-278). Strain selection has resulted in the development of a number of mutant strains of the microorganisms in question which produce a series of desirable compounds including PUFAs. However, the mutation and selection of strains with an improved production of a particular molecule such as the polyunsaturated fatty acids is a time-consuming and difficult process. This is why recombinant methods as described above are preferred whenever possible.

However, only limited amounts of the desired polyunsaturated fatty acids such as DPA, EPA or ARA can be produced with the aid of the abovementioned microorganisms, and, depending on the microorganism used, these are generally obtained as fatty acid mixtures of, for example, EPA, DPA and ARA.

A variety of synthetic pathways is being discussed for the synthesis of arachidonic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (figure 1). Thus, EPA or DHA are produced in marine bacteria such as Vibrio sp. or Shewanella sp. via the polyketide pathway (Yu, R. et al. Lipids 35:1061-1064, 2000; Takeyama, H. et al. Microbiology 143:2725-2731, 1997).

An alternative strategy is the alternating activity of desaturases and elongases (Zank, T.K. et al. Plant Journal 31:255-268, 2002; Sakuradani, E. et al. Gene 238:445-453, 1999). A modification of the above-described pathway by Δ6-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase and Δ4-desaturase is the Sprecher pathway (Sprecher 2000, Biochim. Biophys. Acta 1486:219-231) in mammals. Instead of the Δ4-desaturation, a further elongation step is effected here to give C_{24}, followed by a further Δ6-desaturation and finally β-oxidation to give the C_{22} chain length. Thus what is known as Sprecher pathway (see figure 1) is, however, not suitable for the production in plants and microorganisms since the regulatory mechanisms are not known.

Depending on their desaturation pattern, the polyunsaturated fatty acids can be divided into two large classes, viz. ω6- or ω3-fatty acids, which differ with regard to their metabolic and functional activities (fig. 1).

The starting material for the ω6-metabolic pathway is the fatty acid linoleic acid ($18{:}2^{\Delta 9,12}$) while the ω3-pathway proceeds via linolenic acid ($18{:}3^{\Delta 9,12,15}$). Linolenic acid is formed by the activity of an ω3-desaturase (Tocher et al. 1998, Prog. Lipid Res. 37, 73-117 ; Domergue et al. 2002, Eur. J. Biochem. 269, 4105-4113).

Mammals, and thus also humans, have no corresponding desaturase activity (Δ12- and ω3-desaturase) and must take up these fatty acids (essential fatty acids) via the food. Starting with these precursors, the physiologically important polyunsaturated fatty acids arachidonic acid (= ARA, $20{:}4^{\Delta 5,8,11,14}$), an ω6-fatty acid and the two ω3-fatty acids eicosapentaenoic acid (= EPA, $20{:}5^{\Delta 5,8,11,14,17}$) and docosahexaenoic acid (DHA,

$22:6^{\Delta 4,7,10,13,17,19}$) are synthesized via the sequence of desaturase and elongase reactions. The application of ω3-fatty acids shows the therapeutic activity described above in the treatment of cardiovascular diseases (Shimikawa 2001, World Rev. Nutr. Diet. 88, 100-108), Entzündungen (Calder 2002, Proc. Nutr. Soc. 61, 345-358) and Arthritis (Cleland and James 2000, J. Rheumatol. 27, 2305-2307).

As regards the physiology of nutrition, it is therefore important, when synthesizing polyunsaturated fatty acids, to achieve a shift between the ω6-synthetic pathway and the ω3-synthetic pathway (see figure 1) so that more ω3-fatty acids are produced. The enzymatic activities of a variety of ω3-desaturases which desaturate $C_{18:2}$-, $C_{22:4}$- or $C_{22:5}$-fatty acids have been described in the literature (see figure 1). However, none of the desaturases which have been described in terms of biochemistry converts a broad substrate spectrum of the ω6-synthetic pathway into the corresponding fatty acids of the ω3-synthetic pathway.

There is therefore a continuing high demand for an ω3-desaturase which is suitable for the production of ω3-polyunsaturated fatty acids. All known plant and cyanobacterial ω3-desaturases desaturate C_{18}-fatty acids with linoleic acid as substrate, but cannot desaturate any C_{20}- or C_{22}-fatty acids.

The fungus Saprolegnia dicilina is known to have an ω3-desaturase [Pereira et al. 2004, Biochem. J. 378(Pt 2):665-71] which can desaturate C_{20}-polyunsaturated fatty acids. However, the disadvantage is that this ω3-desaturase cannot desaturate any C_{18}- or C_{22}-PUFAs such as the important fatty acids $C_{18:2}$-, $C_{22:4}$- or $C_{22:5}$-fatty acids of the ω6-synthetic pathway. A further disadvantage of this enzyme is that it cannot desaturate any fatty acids which are bound to phospholipids. Only the CoA-fatty acid esters are converted.

The elongation of fatty acids, by elongases, by 2 or 4 C atoms is of crucial importance for the production of C_{20}- and C_{22}-PUFAs, respectively. This process proceeds via 4 steps. The first step is the condensation of malonyl-CoA with the fatty-acid-acyl-CoA by ketoacyl-CoA synthase (KCS, hereinbelow referred to as elongase). This is followed by a reduction step (ketoacyl-CoA reductase, KCR), a dehydratation step (dehydratase) and a final reduction step (enoyl-CoA reductase). It has been postulated that the elongase activity affects the specificity and rate of the entire process (Millar and Kunst, 1997 Plant Journal 12:121-131).

There have been a large number of attempts in the past to obtain elongase genes. Millar and Kunst, 1997 (Plant Journal 12:121-131) and Millar et al. 1999, (Plant Cell 11:825-838) describe the characterization of plant elongases for the synthesis of monounsaturated long-chain fatty acids (C22:1) and for the synthesis of very long-chain fatty acids for the formation of waxes in plants (C_{28}-C_{32}). Descriptions regarding the synthesis of arachidonic acid and EPA are found, for example, in WO0159128, WO0012720, WO02077213 and WO0208401. The synthesis of polyunsaturated C_{24}-fatty acids is described, for example, in Tvrdik et al. 2000, JCB 149:707-717 or

WO0244320.

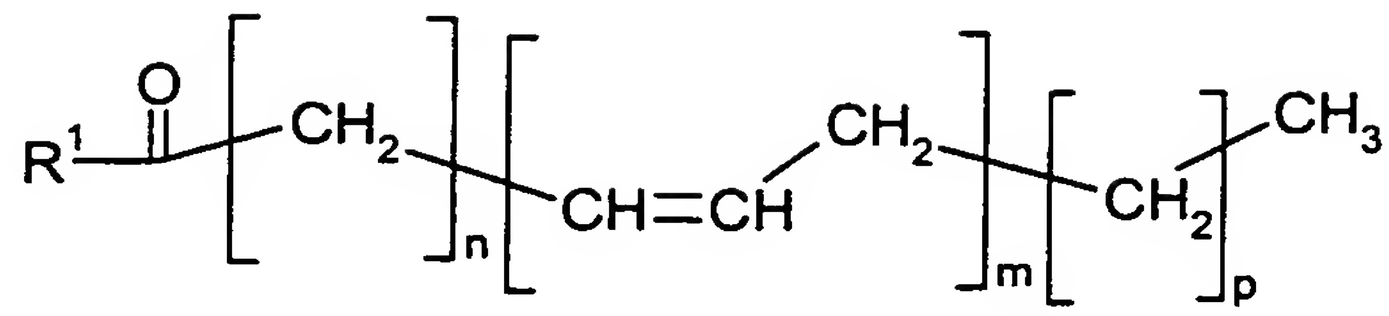
No specific elongase has been described to date for the production of DHA (C22:6 n-3) in organisms which do not naturally produce this fatty acid. Only elongases which provide C_{20}- or C_{24}-fatty acids have been described to date. A Δ5-elongase activity has not been described to date.

Higher plants comprise polyunsaturated fatty acids such as linoleic acid (C18:2) and linolenic acid (C18:3). ARA, EPA and DHA are found not at all in the seed oil of higher plants, or only in miniscule amounts (E. Ucciani: Nouveau Dictionnaire des Huiles Végétales [New Dictionary of Vegetable Oils]. Technique & Documentation – Lavoisier, 1995. ISBN: 2-7430-0009-0). However, the production of LCPUFAs in higher plants, preferably in oil crops such as oilseed rape, linseed, sunflower and soybeans, would be advantageous since large amounts of high-quality LCPUFAs for the food industry, animal nutrition and pharmaceutical purposes might be obtained economically. To this end, it is advantageous to introduce, into oil crops, genes which encode enzymes of the LCPUFA biosynthesis via recombinant methods and to express them therein. These genes encode for example Δ6-desaturases, Δ6-elongases, Δ5-desaturases or Δ4-desaturases. These genes can advantageously be isolated from microorganisms and lower plants which produce LCPUFAs and incorporate them in the membranes or triacylglycerides. Thus, it has already been possible to isolate Δ6-desaturase genes from the moss Physcomitrella patens and Δ6-elongase genes from P. patens and from the nematode C. elegans.

The first transgenic plants which comprise and express genes encoding LCPUFA biosynthesis enzymes and which, as a consequence, produce LCPUFAs were described for the first time, for example, in DE-A-102 19 203 (process for the production of polyunsaturated fatty acids in plants). However, these plants produce LCPUFAs in amounts which require further optimization for processing the oils which are present in the plants.

To make possible the fortification of food and/or of feed with these polyunsaturated fatty acids, there is therefore a great need for a simple, inexpensive process for the production of these polyunsaturated fatty acids, specifically in eukaryotic systems.

It was therefore an object to provide further genes or enzymes which are suitable for the synthesis of LCPUFAs, specifically genes with Δ5-elongase, Δ5-desaturase, Δ4-desaturase, Δ12-desaturase or Δ6-desaturase activity, for the production of polyunsaturated fatty acids. A further object of the present invention was the provision of genes or enzymes which make possible a shift from the ω6-fatty acids to the ω3-fatty acids. Another object was to develop a process for the production of polyunsaturated fatty acids in an organism, advantageously in a eukaryotic organism, preferably in a plant or a microorganism. This object was achieved by the process according to the invention for the production of compounds of the formula I



(I)

in transgenic organisms with a content of at least 1% by weight of these compounds based on the total lipid content of the transgenic organism, which comprises the following process steps:

a) introducing, into the organism, at least one nucleic acid sequence which encodes a Δ9-elongase and/or a Δ6-desaturase activity, and

b) introducing, into the organism, at least one nucleic acid sequence which encodes a Δ8-desaturase and/or a Δ6-elongase activity, and

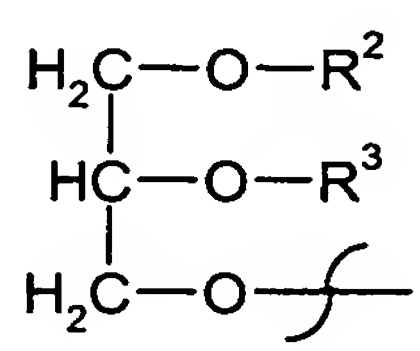
c) introducing, into the organism, at least one nucleic acid sequence which encodes a Δ5-desaturase activity, and

d) introducing, into the organism, at least one nucleic acid sequence which encodes a Δ5-elongase activity, and

e) introducing, into the organism, at least one nucleic acid sequence which encodes a Δ4-desaturase activity, and

where the variables and substituents in formula I have the following meanings:

R^1 = hydroxyl, coenzyme A (thioester), lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol, sphingo base or a radical of the formula II



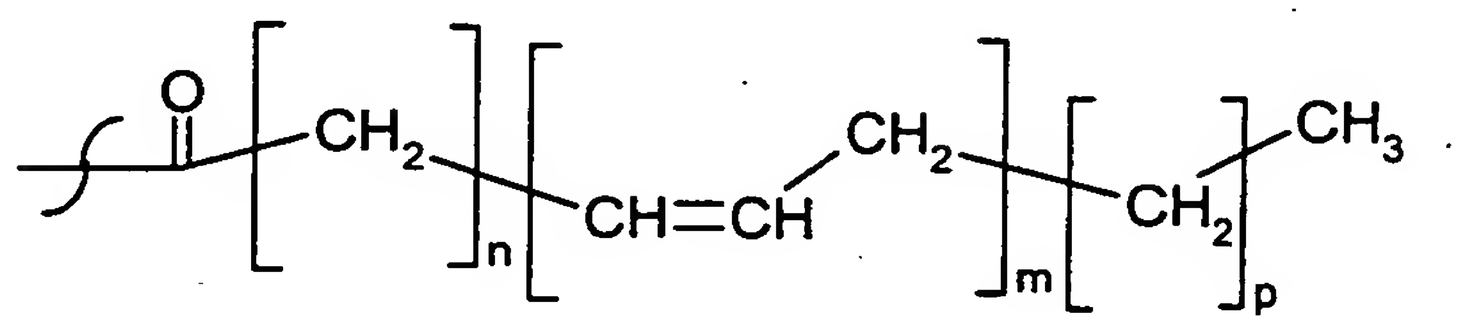
(II)

in which

R^2 = hydrogen, lysophosphatidyl choline, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol or saturated or unsaturated C_2-C_{24}-alkylcarbonyl,

R^3 = hydrogen, saturated or unsaturated C_2-C_{24}-alkylcarbonyl, or R^2 and R^3

independently of one another are a radical of the formula Ia:

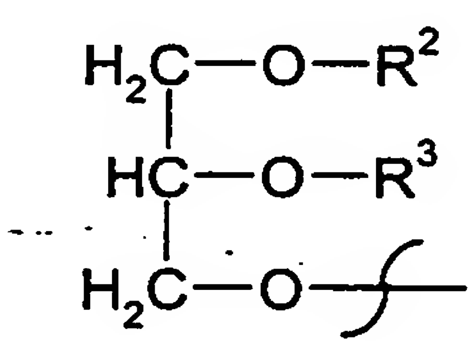


(Ia)

in which

n = 2, 3, 4, 5, 6, 7 or 9, m = 2, 3, 4, 5 or 6 and p = 0 or 3.

R^1 in the formula I is hydroxyl, coenzyme A (thioester), lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol, sphingo base or a radical of the formula II



(II)

The abovementioned radicals of R^1 are always bonded to the compounds of the formula I in the form of their thioesters.

R^2 in the formula II is hydrogen, lysophosphatidylcholine, lysophosphatidylethanolamine, lysophosphatidylglycerol, lysodiphosphatidylglycerol, lysophosphatidylserine, lysophosphatidylinositol or saturated or unsaturated C_2-C_{24}-alkylcarbonyl.

Alkyl radicals which may be mentioned are substituted or unsubstituted, saturated or unsaturated C_2-C_{24}-alkylcarbonyl chains such as ethylcarbonyl, n-propylcarbonyl, n-butylcarbonyl, n-pentylcarbonyl, n-hexylcarbonyl, n-heptylcarbonyl, n-octylcarbonyl, n-nonylcarbonyl, n-decylcarbonyl, n-undecylcarbonyl, n–dodecylcarbonyl, n-tridecyl-carbonyl, n–tetradecylcarbonyl, n–pentadecylcarbonyl, n–hexadecylcarbonyl, n-hepta-decylcarbonyl, n–octadecylcarbonyl-, n–nonadecylcarbonyl, n–eicosylcarbonyl, n-docosanylcarbonyl- or n-tetracosanylcarbonyl, which comprise one or more double bonds. Saturated or unsaturated C_{10}-C_{22}-alkylcarbonyl radicals such as n-decyl-carbonyl, n-undecylcarbonyl, n–dodecylcarbonyl, n–tridecylcarbonyl, n-tetradecyl-carbonyl, n–pentadecylcarbonyl, n–hexadecylcarbonyl, n–heptadecylcarbonyl, n-octadecylcarbonyl, n–nonadecylcarbonyl, n–eicosylcarbonyl, n-docosanylcarbonyl or n-tetracosanylcarbonyl, which comprise one or more double bonds are preferred. Especially preferred are saturated and/or unsaturated C_{10}-C_{22}-alkylcarbonyl radicals such as C_{10}-alkylcarbonyl, C_{11}–alkylcarbonyl, C_{12}–alkylcarbonyl, C_{13}–alkylcarbonyl, C_{14}-alkylcarbonyl, C_{16}–alkylcarbonyl, C_{18}–alkylcarbonyl, C_{20}–alkylcarbonyl or

C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. Very especially preferred are saturated or unsaturated C_{16}-C_{22}-alkylcarbonyl radicals such as C_{16}-alkylcarbonyl, C_{18}-alkylcarbonyl, C_{20}-alkylcarbonyl or C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. These advantageous radicals can comprise two, three, four, five or six double bonds. The especially preferred radicals with 20 or 22 carbon atoms in the fatty acid chain comprise up to six double bonds, advantageously three, four, five or six double bonds, especially preferably five or six double bonds. All the abovementioned radicals are derived from the corresponding fatty acids.

R^3 in the formula II is hydrogen, saturated or unsaturated C_2-C_{24}-alkylcarbonyl.

Alkyl radicals which may be mentioned are substituted or unsubstituted, saturated or unsaturated C_2-C_{24}-alkylcarbonyl chains such as ethylcarbonyl, n-propylcarbonyl, n-butylcarbonyl-, n-pentylcarbonyl, n-hexylcarbonyl, n-heptylcarbonyl, n-octylcarbonyl, n-nonylcarbonyl, n-decylcarbonyl, n-undecylcarbonyl, n–dodecylcarbonyl, n-tridecylcarbonyl, n–tetradecylcarbonyl, n–pentadecylcarbonyl, n–hexadecylcarbonyl, n-heptadecylcarbonyl, n–octadecylcarbonyl-, n–nonadecylcarbonyl, n–eicosylcarbonyl, n-docosanylcarbonyl- or n-tetracosanylcarbonyl, which comprise one or more double bonds. Saturated or unsaturated C_{10}-C_{22}-alkylcarbonyl radicals such as n-decylcarbonyl, n-undecylcarbonyl, n–dodecylcarbonyl, n–tridecylcarbonyl, n-tetradecylcarbonyl, n–pentadecylcarbonyl, n–hexadecylcarbonyl, n–heptadecylcarbonyl, n-octadecylcarbonyl, n–nonadecylcarbonyl, n–eicosylcarbonyl, n-docosanylcarbonyl or n-tetracosanylcarbonyl, which comprise one or more double bonds are preferred. Especially preferred are saturated and/or unsaturated C_{10}-C_{22}-alkylcarbonyl radicals such as C_{10}-alkylcarbonyl, C_{11}–alkylcarbonyl, C_{12}–alkylcarbonyl, C_{13}–alkylcarbonyl, C_{14}-alkylcarbonyl, C_{16}–alkylcarbonyl, C_{18}–alkylcarbonyl, C_{20}–alkylcarbonyl or C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. Very especially preferred are saturated or unsaturated C_{16}-C_{22}-alkylcarbonyl radicals such as C_{16}-alkylcarbonyl, C_{18}-alkylcarbonyl, C_{20}-alkylcarbonyl or C_{22}-alkylcarbonyl radicals which comprise one or more double bonds. These advantageous radicals can comprise two, three, four, five or six double bonds. The especially preferred radicals with 20 or 22 carbon atoms in the fatty acid chain comprise up to six double bonds, advantageously three, four, five or six double bonds, especially preferably five or six double bonds. All the abovementioned radicals are derived from the corresponding fatty acids.

The abovementioned radicals of R^1, R^2 and R^3 can be substituted by hydroxyl and/or epoxy groups and/or can comprise triple bonds.

The polyunsaturated fatty acids produced in the process according to the invention advantageously comprise at least two, advantageously three, four, five or six, double bonds. The fatty acids especially advantageously comprise four, five or six double bonds. Fatty acids produced in the process advantageously have 18, 20 or 22 C atoms in the fatty acid chain; the fatty acids preferably comprise 20 or 22 carbon atoms in the

fatty acid chain. Saturated fatty acids are advantageously reacted to a minor degree, or not at all, by the nucleic acids used in the process. To a minor degree is to be understood as meaning that the saturated fatty acids are reacted with less than 5% of the activity, advantageously less than 3%, especially advantageously with less than 2%, very especially preferably with less than 1, 0.5, 0.25 or 0.125% of the activity in comparison with polyunsaturated fatty acids. These fatty acids which have been produced can be produced in the process as a single product or be present in a fatty acid mixture.

The nucleic acid sequences used in the process according to the invention are isolated nucleic acid sequences which encode polypeptides with Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase and/or Δ4-desaturase activity.

Nucleic acid sequences which are advantageously used in the process according to the invention are those which encode polypeptides with Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183, or

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72,

SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132 or SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138 or SEQ ID NO: 184, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183, which encode polypeptides with at least 40% identity at the amino acid level with SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, , SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132 or SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138 or SEQ ID NO: 184 or and which have Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase activity.

The substituents R^2 or R^3 in the formulae I and II are advantageously and independently of one another saturated or unsaturated C_{18}-C_{22}-alkylcarbonyl, especially advantageously they are, independently of one another, unsaturated C_{18}-,

C_{20}- or C_{22}-alkylcarbonyl with at least two double bonds.

A preferred embodiment of the method is characterized in that a nucleic acid sequence which encodes polypeptides with ω3-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 87 or SEQ ID NO: 105, or

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 88 or SEQ ID NO: 106, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 87 or SEQ ID NO: 105 which encode polypeptides with at least 60% identity at the amino acid level with SEQ ID NO: 88 or SEQ ID NO: 106 and which have ω3-desaturase activity.

is additionally introduced into the organism.

In a further preferred embodiment, the process comprises the additional introduction, into the organism, of a nucleic acid sequence which encodes polypeptides with Δ12-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 107 or SEQ ID NO: 109 or

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 108 or SEQ ID NO: 110, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 107 or SEQ ID NO: 109 which encode polypeptides with at least 60% identity at the amino acid level with SEQ ID NO: 108 or SEQ ID NO: 110 and which have Δ12-desaturase activity.

These abovementioned Δ12-desaturase sequences can be used together with the nucleic acid sequences used in the process and which encode Δ9-elongases, Δ6-desaturases, Δ8-desaturases, Δ6-elongases, Δ5-desaturases, Δ5-elongases and/or Δ4-desaturases, alone or in combination with the ω3-desaturase sequences.

Table 1 shows the nucleic acid sequences, the organism of origin and the sequence ID number.

No.	Organism	Activity	Sequence number
1.	Euglena gracilis	Δ8-desaturase	SEQ ID NO: 1
2.	Isochrysis galbana	Δ9-elongase	SEQ ID NO: 3
3.	Phaedodactylum tricornutum	Δ5-desaturase	SEQ ID NO: 5
4.	Ceratodon pupureus	Δ5-desaturase	SEQ ID NO: 7
5.	Physcomitrella patens	Δ5-desaturase	SEQ ID NO: 9
6.	Thraustrochytrium sp.	Δ5-desaturase	SEQ ID NO: 11
7.	Mortierella alpina	Δ5-desaturase	SEQ ID NO: 13
8.	Caenorhabditis elegans	Δ5-desaturase	SEQ ID NO: 15
9.	Borago officinalis	Δ6-desaturase	SEQ ID NO: 17
10.	Ceratodon purpureus	Δ6-desaturase	SEQ ID NO: 19
11.	Phaeodactylum tricornutum	Δ6-desaturase	SEQ ID NO: 21
12.	Physcomitrella patens	Δ6-desaturase	SEQ ID NO: 23
13.	Caenorhabditis elegans	Δ6-desaturase	SEQ ID NO: 25
14.	Physcomitrella patens	Δ6-elongase	SEQ ID NO: 27
15.	Thraustrochytrium sp.	Δ6-elongase	SEQ ID NO: 29
16.	Phytopthera infestans	Δ6-elongase	SEQ ID NO: 31
17.	Mortierella alpina	Δ6-elongase	SEQ ID NO: 33
18.	Mortierella alpina	Δ6-elongase	SEQ ID NO: 35
19.	Caenorhabditis elegans	Δ6-elongase	SEQ ID NO: 37
20.	Euglena gracilis	Δ4-desaturase	SEQ ID NO: 39
21.	Thraustrochytrium sp.	Δ4-desaturase	SEQ ID NO: 41
22.	Thalassiosira pseudonana	Δ5-elongase	SEQ ID NO: 43
23.	Thalassiosira pseudonana	Δ6-elongase	SEQ ID NO: 45
24.	Crypthecodinium cohnii	Δ5-elongase	SEQ ID NO: 47
25.	Crypthecodinium cohnii	Δ5-elongase	SEQ ID NO: 49
26.	Oncorhynchus mykiss	Δ5-elongase	SEQ ID NO: 51
27.	Oncorhynchus mykiss	Δ5-elongase	SEQ ID NO: 53
28.	Thalassiosira pseudonana	Δ5-elongase	SEQ ID NO: 59
29.	Thalassiosira pseudonana	Δ5-elongase	SEQ ID NO: 61

No.	Organism	Activity	Sequence number
30.	Thalassiosira pseudonana	Δ5-elongase	SEQ ID NO: 63
31.	Thraustrochytrium aureum	Δ5-elongase	SEQ ID NO: 65
32.	Ostreococcus tauri	Δ5-elongase	SEQ ID NO: 67
33.	Ostreococcus tauri	Δ6-elongase	SEQ ID NO: 69
34.	Primula farinosa	Δ6-desaturase	SEQ ID NO: 71
35.	Primula vialii	Δ6-desaturase	SEQ ID NO: 73
36.	Ostreococcus tauri	Δ5-elongase	SEQ ID NO: 75
37.	Ostreococcus tauri	Δ5-elongase	SEQ ID NO: 77
38.	Ostreococcus tauri	Δ5-elongase	SEQ ID NO: 79
39.	Ostreococcus tauri	Δ6-elongase	SEQ ID NO: 81
40.	Thraustrochytrium sp.	Δ5-elongase	SEQ ID NO: 83
41.	Thalassiosira pseudonana	Δ5-elongase	SEQ ID NO: 85
42.	Phytopthora infestans	ω3-desaturase	SEQ ID NO: 87
43.	Ostreococcus tauri	Δ6-desaturase	SEQ ID NO: 89
44.	Ostreococcus tauri	Δ5-desaturase	SEQ ID NO: 91
45.	Ostreococcus tauri	Δ5-desaturase	SEQ ID NO: 93
46.	Ostreococcus tauri	Δ4-desaturase	SEQ ID NO: 95
47.	Thalassiosira pseudonana	Δ6-desaturase	SEQ ID NO: 97
48.	Thalassiosira pseudonana	Δ5-desaturase	SEQ ID NO: 99
49.	Thalassiosira pseudonana	Δ5-desaturase	SEQ ID NO: 101
50.	Thalassiosira pseudonana	Δ4-desaturase	SEQ ID NO: 103
51.	Thalassiosira pseudonana	ω3-desaturase	SEQ ID NO: 105
52.	Ostreococcus tauri	Δ12-desaturase	SEQ ID NO: 107
53.	Thalassiosira pseudonana	Δ12-desaturase	SEQ ID NO: 109
54.	Ostreococcus tauri	Δ6-elongase	SEQ ID NO: 111
55.	Ostreococcus tauri	Δ5-elongase	SEQ ID NO: 113
56.	Xenopus laevis (BC044967)	Δ5-elongase	SEQ ID NO: 117
57.	Ciona intestinalis (AK112719)	Δ5-elongase	SEQ ID NO: 119
58.	Euglena gracilis	Δ5-elongase	SEQ ID NO: 131
59.	Euglena gracilis	Δ5-elongase	SEQ ID NO: 133

No.	Organism	Activity	Sequence number
60.	Arabidopsis thaliana	Δ5-elongase	SEQ ID NO: 135
61.	Arabidopsis thaliana	Δ5-elongase	SEQ ID NO: 137
62.	Phaeodactylum tricornutum	Δ6-elongase	SEQ ID NO: 183

The polyunsaturated fatty acids produced in the process are advantageously bound in membrane lipids and/or triacylglycerides, but may also occur in the organisms as free fatty acids or else bound in the form of other fatty acid esters. In this context, they may be present as "pure products" or else advantageously in the form of mixtures of various fatty acids or mixtures of different glycerides. The various fatty acids which are bound in the triacylglycerides can be derived from short-chain fatty acids with 4 to 6 C atoms, medium-chain fatty acids with 8 to 12 C atoms or long-chain fatty acids with 14 to 24 C atoms, preferred are long-chain fatty acids more preferably long-chain polyunsaturated fatty acids with 18, 20 and/or 22 C atoms.

The process according to the invention advantageously yields fatty acid esters with polyunsaturated C_{18}–, C_{20}- and/or C_{22}-fatty acid molecules with at least two double bonds in the fatty acid ester, advantageously with at least three, four, five or six double bonds in the fatty acid ester, especially advantageously with at least five or six double bonds in the fatty acid ester and advantageously leads to the synthesis of linoleic acid (=LA, $C18:2^{\Delta 9,12}$), γ-linolenic acid (= GLA, $C18:3^{\Delta 6,9,12}$), stearidonic acid (= SDA, $C18:4^{\Delta 6,9,12,15}$), dihomo-γ-linolenic acid (= DGLA, $20:3^{\Delta 8,11,14}$), ω3-eicosatetraenoic acid (= ETA, $C20:4^{\Delta 5,8,11,14}$), arachidonic acid (ARA, $C20:4^{\Delta 5,8,11,14}$), eicosapentaenoic acid (EPA, $C20:5^{\Delta 5,8,11,14,17}$), ω6-docosapentaenoic acid ($C22:5^{\Delta 4,7,10,13,16}$), ω6-docosatetraenoic acid ($C22:4^{\Delta,7,10,13,16}$), ω3-docosapentaenoic acid (= DPA, $C22:5^{\Delta 7,10,13,16,19}$), docosahexaenoic acid (= DHA, $C22:6^{\Delta 4,7,10,13,16,19}$) or mixtures of these, preferably ARA, EPA and/or DHA. ω3-Fatty acids such as EPA and/or DHA are very especially preferably produced.

The fatty acid esters with polyunsaturated C_{18}–, C_{20}- and/or C_{22}-fatty acid molecules can be isolated in the form of an oil or lipid, for example in the form of compounds such as sphingolipids, phosphoglycerides, lipids, glycolipids such as glycosphingolipids, phospholipids such as phosphatidylethanolamine, phosphatidylcholine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol or diphosphatidylglycerol, monoacylglycerides, diacylglycerides, triacylglycerides or other fatty acid esters such as the acetyl-coenzyme A esters which comprise the polyunsaturated fatty acids with at least two, three, four, five or six, preferably five or six double bonds, from the organisms which have been used for the preparation of the fatty acid esters; preferably, they are isolated in the form of their diacylglycerides, triacylglycerides and/or in the form of phosphatidylcholine, especially preferably in the form of the triacylglycerides. In addition to these esters, the polyunsaturated fatty acids are also present in the organisms, advantageously the plants as free fatty acids or bound in other compounds. As a rule, the various abovementioned compounds (fatty acid esters and free fatty

acids) are present in the organisms with an approximate distribution of 80 to 90% by weight of triglycerides, 2 to 5% by weight of diglycerides, 5 to 10% by weight of monoglycerides, 1 to 5% by weight of free fatty acids, 2 to 8% by weight of phospholipids, the total of the various compounds amounting to 100% by weight.

The process according to the invention yields the LCPUFAs produced in a content of at least 3% by weight, advantageously at least 5% by weight, preferably at least 8% by weight, especially preferably at least 10% by weight, most preferably at least 15% by weight, based on the total fatty acids in the transgenic organisms, preferably in a transgenic plant. In this context, it is advantageous to convert C_{18}- and/or C_{20}-fatty acids which are present in the host organisms to at least 10%, preferably to at least 20%, especially preferably to at least 30%, most preferably to at least 40% to give the corresponding products such as DPA or DHA, to mention just two examples. The fatty acids are advantageously produced in bound form. These unsaturated fatty acids can, with the aid of the nucleic acids used in the process according to the invention, be positioned at the sn1, sn2 and/or sn3 position of the advantageously produced triglycerides. Since a plurality of reaction steps are performed by the starting compounds linoleic acid (C18:2) and linolenic acid (C18:3) in the process according to the invention, the end products of the process such as, for example, arachidonic acid (ARA), eicosapentaenoic acid (EPA) ω6-docosapentaenoic acid or DHA are not obtained as absolutely pure products; minor traces of the precursors are always present in the end product. If, for example, both linoleic acid and linolenic acid are present in the starting organism and the starting plant, the end products such as ARA, EPA or DHA are present as mixtures. The precursors should advantageously not amount to more than 20% by weight, preferably not to more than 15% by weight, especially preferably not to more than 10% by weight, most preferably not to more than 5% by weight, based on the amount of the end product in question. Advantageously, only ARA, EPA or only DHA, bound or as free acids, are produced as end products in a transgenic plant owing to the process according to the invention. If the compounds ARA, EPA and DHA are produced simultaneously, they are advantageously produced in a ratio of at least 1:1:2 (EPA:ARA:DHA), advantageously of at least 1:1:3, preferably 1:1:4, especially preferably 1:1:5.

Fatty acid esters or fatty acid mixtures produced by the process according to the invention advantageously comprise 6 to 15% of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. Advantageous polyunsaturated fatty acids which are present in the fatty acid esters or fatty acid mixtures are preferably at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1% of arachidonic acid, based on the total fatty acid content. Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the process of the invention advantageously comprise fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methylene-octadec-9-enoic acid), malvalic acid (8,9-methyleneheptadec-8-enoic acid),

chaulmoogric acid (cyclopentenedodecanoic acid), furan fatty acid (9,12-epoxyocta-deca-9,11-dienoic acid), vernolic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10-heptadecen-8-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12t-octa-decadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid), catalpic acid (9t11t13c-octadecatrienoic acid), eleostearic acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadecatrienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid), pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienoic acid). The abovementioned fatty acids are, as a rule, advantageously only found in traces in the fatty acid esters or fatty acid mixtures produced by the process according to the invention, that is to say that, based on the total fatty acids, they occur to less than 30%, preferably to less than 25%, 24%, 23%, 22% or 21%, especially preferably to less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very especially preferably to less than 4%, 3%, 2% or 1%. In a further preferred form of the invention, these abovementioned fatty acids occur to less than 0.9%, 0.8%, 0.7%, 0.6% or 0.5%, especially preferably to less than 0.4%, 0.3%, 0.2%, 0.1%, based on the total fatty acids. The fatty acid esters or fatty acid mixtures produced by the process according to the invention advantageously comprise less than 0.1%, based on the total fatty acids, and/or no butyric acid, no cholesterol, no clupanodonic acid (= docosapentaenoic acid, $C22{:}5^{\Delta 4,8,12,15,21}$) and no nisinic acid (tetracosahexaenoic acid, $C23{:}6^{\Delta 3,8,12,15,18,21}$).

Owing to the nucleic acid sequences, or the nucleic acid sequences used in the process according to the invention, an increase in the yield of polyunsaturated fatty acids of at least 50%, advantageously of at least 80%, especially advantageously of at least 100%, very especially advantageously of at least 150%, in comparison with the nontransgenic starting organism, for example a yeast, an alga, a fungus or a plant such as arabidopsis or linseed can be obtained when the fatty acids are detected by GC analysis (see examples).

Chemically pure polyunsaturated fatty acids or fatty acid compositions can also be synthesized by the processes described above. To this end, the fatty acids or the fatty acid compositions are isolated from the organisms, such as the microorganisms or the plants or the culture medium in or on which the organisms have been grown, or from the organism and the culture medium, in the known manner, for example via extraction, distillation, crystallization, chromatography or a combination of these methods. These chemically pure fatty acids or fatty acid compositions are advantageous for applications in the food industry sector, the cosmetic sector and especially the pharmacological industry sector.

Suitable organisms for the production in the process according to the invention are, in principle, any organisms such as microorganisms, nonhuman animals or plants.

Plants which are suitable are, in principle, all those plants which are capable of synthesizing fatty acids, such as all dicotyledonous or monocotyledonous plants, algae or mosses. Advantageous plants are selected from the group of the plant families Adelotheciaceae, Anacardiaceae, Asteraceae, Apiaceae, Betulaceae, Boraginaceae, Brassicaceae, Bromeliaceae, Caricaceae, Cannabaceae, Convolvulaceae, Chenopodiaceae, Crypthecodiniaceae, Cucurbitaceae, Ditrichaceae, Elaeagnaceae, Ericaceae, Euphorbiaceae, Fabaceae, Geraniaceae, Gramineae, Juglandaceae, Lauraceae, Leguminosae, Linaceae, Euglenaceae, Prasinophyceae or vegetable plants or ornamentals such as Tagetes.

Examples which may be mentioned are the following plants selected from the group consisting of: Adelotheciaceae such as the genera Physcomitrella, for example the genus and species *Physcomitrella patens,* Anacardiaceae such as the genera Pistacia, Mangifera, Anacardium, for example the genus and species *Pistacia vera* [pistachio], *Mangifer indica* [mango] or *Anacardium occidentale* [cashew], Asteraceae, such as the genera Calendula, Carthamus, Centaurea, Cichorium, Cynara, Helianthus, Lactuca, Locusta, Tagetes, Valeriana, for example the genus and species Calendula officinalis [common marigold], Carthamus tinctorius [safflower], *Centaurea cyanus* [cornflower], *Cichorium intybus* [chicory], Cynara scolymus [artichoke], *Helianthus annus* [sunflower], *Lactuca sativa, Lactuca crispa, Lactuca esculenta, Lactuca scariola L. ssp. sativa, Lactuca scariola L. var. integrata, Lactuca scariola L. var. integrifolia, Lactuca sativa subsp. romana*, *Locusta communis, Valeriana locusta* [salad vegetables], *Tagetes lucida, Tagetes erecta* or *Tagetes tenuifolia* [african or french marigold], Apiaceae, such as the genus Daucus, for example the genus and species *Daucus carota* [carrot], Betulaceae, such as the genus Corylus, for example the genera and species *Corylus avellana* or *Corylus colurna* [hazelnut], Boraginaceae, such as the genus Borago, for example the genus and species *Borago officinalis* [borage], Brassicaceae, such as the genera Brassica, Camelina, Melanosinapis, Sinapis, Arabadopsis, for example the genera and species *Brassica napus*, *Brassica rapa* ssp. [oilseed rape], *Sinapis arvensis Brassica juncea, Brassica juncea var. juncea, Brassica juncea var. crispifolia, Brassica juncea var. foliosa, Brassica nigra, Brassica sinapioides,* Camelina sativa, *Melanosinapis communis* [mustard], *Brassica oleracea* [fodder beet] or Arabidopsis thaliana, Bromeliaceae, such as the genera Anana, Bromelia (pineapple), for example the genera and species *Anana comosus, Ananas ananas* or *Bromelia comosa* [pineapple], Caricaceae, such as the genus Carica, such as the genus and species *Carica papaya* [pawpaw], Cannabaceae, such as the genus Cannabis, such as the genus and species *Cannabis sativa* [hemp], Convolvulaceae, such as the genera Ipomea, Convolvulus, for example the genera and species *Ipomoea batatus, Ipomoea pandurata, Convolvulus batatas, Convolvulus tiliaceus, Ipomoea fastigiata, Ipomoea tiliacea, Ipomoea triloba* or *Convolvulus panduratus* [sweet potato, batate], Chenopodiaceae, such as the genus Beta, such as the genera and species *Beta vulgaris, Beta vulgaris var. altissima*, *Beta vulgaris var. vulgaris, Beta maritima, Beta vulgaris var. perennis, Beta vulgaris var. conditiva* or *Beta vulgaris var. esculenta* [sugarbeet], Crypthecodiniaceae, such as the genus Crypthecodinium, for

example the genus and species *Cryptecodinium cohnii,* Cucurbitaceae, such as the genus Cucurbita, for example the genera and species *Cucurbita maxima, Cucurbita mixta, Cucurbita pepo* or *Cucurbita moschata* [pumpkin/squash], Cymbellaceae, such as the genera Amphora, Cymbella, Okedenia, Phaeodactylum, Reimeria, for example the genus and species *Phaeodactylum tricornutum,* Ditrichaceae, such as the genera Ditrichaceae, Astomiopsis, Ceratodon, Chrysoblastella, Ditrichum, Distichium, Eccremidium, Lophidion, Philibertiella, Pleuridium, Saelania, Trichodon, Skottsbergia, for example the genera and species *Ceratodon antarcticus, Ceratodon columbiae, Ceratodon heterophyllus, Ceratodon purpurascens, Ceratodon purpureus, Ceratodon purpureus ssp. convolutus, Ceratodon purpureus ssp. stenocarpus, Ceratodon purpureus var. rotundifolius, Ceratodon ratodon, Ceratodon stenocarpus, Chrysoblastella chilensis, Ditrichum ambiguum, Ditrichum brevisetum, Ditrichum crispatissimum, Ditrichum difficile, Ditrichum falcifolium, Ditrichum flexicaule, Ditrichum giganteum, Ditrichum heteromallum, Ditrichum lineare, Ditrichum lineare, Ditrichum montanum, Ditrichum montanum, Ditrichum pallidum, Ditrichum punctulatum, Ditrichum pusillum, Ditrichum pusillum var. tortile, Ditrichum rhynchostegium, Ditrichum schimperi, Ditrichum tortile, Distichium capillaceum, Distichium hagenii, Distichium inclinatum, Distichium macounii, Eccremidium floridanum, Eccremidium whiteleggei, Lophidion strictus, Pleuridium acuminatum, Pleuridium alternifolium, Pleuridium holdridgei, Pleuridium mexicanum, Pleuridium ravenelii, Pleuridium subulatum, Saelania glaucescens, Trichodon borealis, Trichodon cylindricus or Trichodon cylindricus var. oblongus,* Elaeagnaceae, such as the genus Elaeagnus, for example the genus and species *Olea europaea* [olive], Ericaceae, such as the genus Kalmia, for example the genera and species *Kalmia latifolia, Kalmia angustifolia, Kalmia microphylla, Kalmia polifolia, Kalmia occidentalis, Cistus chamaerhodendros* or *Kalmia lucida* [mountain laurel], Euglenaceae, such as the genera Ascoglena, Astasia, Colacium, Cyclidiopsis, Euglena, Euglenopsis, Hyalaphacus, Khawkinea, Lepocinclis, Phacus, Strombomonas, Trachelomonas, for example the genus and species *Euglena gracilis*; Euphorbiaceae, such as the genera Manihot, Janipha, Jatropha, Ricinus, for example the genera and species *Manihot utilissima, Janipha manihot, Jatropha manihot, Manihot aipil, Manihot dulcis, Manihot manihot, Manihot melanobasis, Manihot esculenta* [cassava] or *Ricinus communis* [castor-oil plant], Fabaceae, such as the genera Pisum, Albizia, Cathormion, Feuillea, Inga, Pithecolobium, Acacia, Mimosa, Medicajo, Glycine, Dolichos, Phaseolus, soybean, for example the genera and species *Pisum sativum, Pisum arvense, Pisum humile* [pea], *Albizia berteriana, Albizia julibrissin, Albizia lebbeck, Acacia berteriana, Acacia littoralis, Albizia berteriana, Albizzia berteriana, Cathormion berteriana, Feuillea berteriana, Inga fragrans, Pithecellobium berterianum, Pithecellobium fragrans, Pithecolobium berterianum, Pseudalbizzia berteriana, Acacia julibrissin, Acacia nemu, Albizia nemu, Feuilleea julibrissin, Mimosa julibrissin, Mimosa speciosa, Sericanrda julibrissin, Acacia lebbeck, Acacia macrophylla, Albizia lebbeck, Feuilleea lebbeck, Mimosa lebbeck, Mimosa speciosa, Medicago sativa, Medicago falcata, Medicago varia* [alfalfa] *Glycine max Dolichos soja, Glycine gracilis, Glycine hispida, Phaseolus max, Soja hispida* or *Soja max* [soybean], Funariaceae, such as the genera Aphanorrhegma, Entosthodon,

Funaria, Physcomitrella, Physcomitrium, for example the genera and species *Aphanorrhegma serratum, Entosthodon attenuatus, Entosthodon bolanderi, Entosthodon bonplandii, Entosthodon californicus, Entosthodon drummondii, Entosthodon jamesonii, Entosthodon leibergii, Entosthodon neoscoticus, Entosthodon rubrisetus, Entosthodon spathulifolius, Entosthodon tucsoni, Funaria americana, Funaria bolanderi, Funaria calcarea, Funaria californica, Funaria calvescens, Funaria convoluta, Funaria flavicans, Funaria groutiana, Funaria hygrometrica, Funaria hygrometrica var. arctica, Funaria hygrometrica var. calvescens, Funaria hygrometrica var. convoluta, Funaria hygrometrica var. muralis, Funaria hygrometrica var. utahensis, Funaria microstoma, Funaria microstoma var. obtusifolia, Funaria muhlenbergii, Funaria orcuttii, Funaria plano-convexa, Funaria polaris, Funaria ravenelii, Funaria rubriseta, Funaria serrata, Funaria sonorae, Funaria sublimbatus, Funaria tucsoni, Physcomitrella californica, Physcomitrella patens, Physcomitrella readeri, Physcomitrium australe, Physcomitrium californicum, Physcomitrium collenchymatum, Physcomitrium coloradense, Physcomitrium cupuliferum, Physcomitrium drummondii, Physcomitrium eurystomum, Physcomitrium flexifolium, Physcomitrium hookeri, Physcomitrium hookeri var. serratum, Physcomitrium immersum, Physcomitrium kellermanii, Physcomitrium megalocarpum, Physcomitrium pyriforme, Physcomitrium pyriforme var. serratum, Physcomitrium rufipes, Physcomitrium sandbergii, Physcomitrium subsphaericum, Physcomitrium washingtoniense,* Geraniaceae, such as the genera Pelargonium, Cocos, Oleum, for example the genera and species *Cocos nucifera, Pelargonium grossularioides* or *Oleum cocois* [coconut], Gramineae, such as the genus Saccharum, for example the genus and species Saccharum officinarum, Juglandaceae, such as the genera Juglans, Wallia, for example the genera and species *Juglans regia, Juglans ailanthifolia, Juglans sieboldiana, Juglans cinerea, Wallia cinerea, Juglans bixbyi, Juglans californica, Juglans hindsii, Juglans intermedia, Juglans jamaicensis, Juglans major, Juglans microcarpa, Juglans nigra* or *Wallia nigra* [walnut], Lauraceae, such as the genera Persea, Laurus, for example the genera and species *Laurus nobilis* [bay], *Persea americana, Persea gratissima* or *Persea persea* [avocado], Leguminosae, such as the genus Arachis, for example the genus and species *Arachis hypogaea* [peanut], Linaceae, such as the genera Adenolinum, for example the genera and species *Linum usitatissimum, Linum humile, Linum austriacum, Linum bienne, Linum angustifolium, Linum catharticum, Linum flavum, Linum grandiflorum, Adenolinum grandiflorum, Linum lewisii, Linum narbonense, Linum perenne, Linum perenne var. lewisii, Linum pratense* or *Linum trigynum* [linseed], Lythrarieae, such as the genus Punica, for example the genus and species *Punica granatum* [pomegranate], Malvaceae, such as the genus Gossypium, for example the genera and species *Gossypium hirsutum, Gossypium arboreum, Gossypium barbadense, Gossypium herbaceum* or *Gossypium thurberi* [cotton], Marchantiaceae, such as the genus Marchantia, for example the genera and species *Marchantia berteroana, Marchantia foliacea, Marchantia macropora,* Musaceae, such as the genus Musa, for example the genera and species *Musa nana, Musa acuminata, Musa paradisiaca, Musa* spp. [banana], Onagraceae, such as the genera Camissonia, Oenothera, for example the genera and species *Oenothera biennis* or *Camissonia*

brevipes [evening primrose], Palmae, such as the genus Elaeis, for example the genus and species *Elaeis guineensis* [oil palm], Papaveraceae, such as, for example, the genus Papaver, for example the genera and species *Papaver orientale, Papaver rhoeas, Papaver dubium* [poppy], Pedaliaceae, such as the genus Sesamum, for example the genus and species *Sesamum indicum* [sesame], Piperaceae, such as the genera Piper, Artanthe, Peperomia, Steffensia, for example the genera and species *Piper aduncum, Piper amalago, Piper angustifolium, Piper auritum, Piper betel, Piper cubeba, Piper longum, Piper nigrum, Piper retrofractum, Artanthe adunca, Artanthe elongata, Peperomia elongata, Piper elongatum, Steffensia elongata* [cayenne pepper], Poaceae, such as the genera Hordeum, Secale, Avena, Sorghum, Andropogon, Holcus, Panicum, Oryza, Zea (maize), Triticum, for example the genera and species *Hordeum vulgare, Hordeum jubatum, Hordeum murinum, Hordeum secalinum, Hordeum distichon Hordeum aegiceras, Hordeum hexastichon, Hordeum hexastichum, Hordeum irregulare, Hordeum sativum, Hordeum secalinum* [barley], *Secale cereale* [rye], Avena sativa, *Avena fatua, Avena byzantina, Avena fatua var. sativa, Avena hybrida* [oats], *Sorghum bicolor, Sorghum halepense, Sorghum saccharatum, Sorghum vulgare, Andropogon drummondii, Holcus bicolor, Holcus sorghum, Sorghum aethiopicum, Sorghum arundinaceum, Sorghum caffrorum, Sorghum cernuum, Sorghum dochna, Sorghum drummondii, Sorghum durra, Sorghum guineense, Sorghum lanceolatum, Sorghum nervosum, Sorghum saccharatum, Sorghum subglabrescens, Sorghum verticilliflorum, Sorghum vulgare, Holcus halepensis, Sorghum miliaceum, Panicum militaceum [millet], Oryza sativa, Oryza latifolia* [rice], *Zea mays* [maize] *Triticum aestivum, Triticum durum, Triticum turgidum, Triticum hybernum, Triticum macha, Triticum sativum* or *Triticum vulgare* [wheat], Porphyridiaceae, such as the genera Chroothece, Flintiella, Petrovanella, Porphyridium, Rhodella, Rhodosorus, Vanhoeffenia, for example the genus and species *Porphyridium cruentum,* Proteaceae, such as the genus Macadamia, for example the genus and species *Macadamia intergrifolia* [macadamia], Prasinophyceae, such as the genera Nephroselmis, Prasinococcus, Scherffelia, Tetraselmis, Mantoniella, Ostreococcus, for example the genera and species *Nephroselmis olivacea, Prasinococcus capsulatus, Scherffelia dubia, Tetraselmis chui, Tetraselmis suecica,* Mantoniella squamata, Ostreococcus tauri, Rubiaceae, such as the genus Coffea, for example the genera and species Coffea spp., *Coffea arabica, Coffea canephora* or *Coffea liberica* [coffee], Scrophulariaceae, such as the genus Verbascum, for example the genera and species *Verbascum blattaria, Verbascum chaixii, Verbascum densiflorum, Verbascum lagurus, Verbascum longifolium, Verbascum lychnitis, Verbascum nigrum, Verbascum olympicum, Verbascum phlomoides, Verbascum phoenicum, Verbascum pulverulentum* or *Verbascum thapsus* [verbascum], Solanaceae, such as the genera Capsicum, Nicotiana, Solanum, Lycopersicon, for example the genera and species *Capsicum annuum, Capsicum annuum var. glabriusculum, Capsicum frutescens* [pepper], *Capsicum annuum* [paprika], *Nicotiana tabacum, Nicotiana alata, Nicotiana attenuata, Nicotiana glauca, Nicotiana langsdorffii, Nicotiana obtusifolia, Nicotiana quadrivalvis, Nicotiana repanda, Nicotiana rustica, Nicotiana sylvestris* [tobacco], *Solanum tuberosum* [potato], *Solanum*

melongena [eggplant] *Lycopersicon esculentum, Lycopersicon lycopersicum, Lycopersicon pyriforme, Solanum integrifolium* or *Solanum lycopersicum* [tomato], Sterculiaceae, such as the genus Theobroma, for example the genus and species *Theobroma cacao* [cacao] or Theaceae, such as the genus Camellia, for example the genus and species *Camellia sinensis* [tea].

Advantageous microorganisms are, for example, fungi selected from the group of the families Chaetomiaceae, Choanephoraceae, Cryptococcaceae, Cunninghamellaceae, Demetiaceae, Moniliaceae, Mortierellaceae, Mucoraceae, Pythiaceae, Sacharomycetaceae, Saprolegniaceae, Schizosacharomycetaceae, Sodariaceae or Tuberculariaceae.

Examples of microorganisms which may be mentioned are those from the groups: Choanephoraceae, such as the genera Blakeslea, Choanephora, for example the genera and species *Blakeslea trispora, Choanephora cucurbitarum, Choanephora infundibulifera* var. *cucurbitarum,* Mortierellaceae, such as the genus Mortierella, for example the genera and species *Mortierella isabellina, Mortierella polycephala* , *Mortierella ramanniana* , *Mortierella vinacea, Mortierella zonata,* Pythiaceae, such as the genera Phytium, Phytophthora, for example the genera and species *Pythium debaryanum, Pythium intermedium, Pythium irregulare, Pythium megalacanthum, Pythium paroecandrum, Pythium sylvaticum, Pythium ultimum, Phytophthora cactorum, Phytophthora cinnamomi, Phytophthora citricola, Phytophthora citrophthora, Phytophthora cryptogea, Phytophthora drechsleri, Phytophthora erythroseptica, Phytophthora lateralis, Phytophthora megasperma, Phytophthora nicotianae, Phytophthora nicotianae var. parasitica, Phytophthora palmivora, Phytophthora parasitica, Phytophthora syringae*, Saccharomycetaceae, such as the genera Hansenula, Pichia, Saccharomyces, Saccharomycodes, Yarrowia, for example the genera and species *Hansenula anomala, Hansenula californica, Hansenula canadensis, Hansenula capsulata, Hansenula ciferrii, Hansenula glucozyma, Hansenula henricii, Hansenula holstii, Hansenula minuta, Hansenula nonfermentans, Hansenula philodendri, Hansenula polymorpha, Hansenula saturnus, Hansenula subpelliculosa, Hansenula wickerhamii, Hansenula wingei, Pichia alcoholophila, Pichia angusta, Pichia anomala, Pichia bispora, Pichia burtonii, Pichia canadensis, Pichia capsulata, Pichia carsonii, Pichia cellobiosa, Pichia ciferrii, Pichia farinosa, Pichia fermentans, Pichia finlandica, Pichia glucozyma, Pichia guilliermondii, Pichia haplophila, Pichia henricii, Pichia holstii, Pichia jadinii, Pichia lindnerii, Pichia membranaefaciens, Pichia methanolica, Pichia minuta var. minuta, Pichia minuta var. nonfermentans, Pichia norvegensis, Pichia ohmeri, Pichia pastoris, Pichia philodendri, Pichia pini, Pichia polymorpha, Pichia quercuum, Pichia rhodanensis, Pichia sargentensis, Pichia stipitis, Pichia strasburgensis, Pichia subpelliculosa, Pichia toletana, Pichia trehalophila, Pichia vini, Pichia xylosa, Saccharomyces aceti, Saccharomyces bailii, Saccharomyces bayanus, Saccharomyces bisporus, Saccharomyces capensis, Saccharomyces carlsbergensis, Saccharomyces cerevisiae, Saccharomyces cerevisiae var. ellipsoideus, Saccharomyces chevalieri, Saccharomyces delbrueckii, Saccharomyces diastaticus, Saccharomyces*

drosophilarum, Saccharomyces elegans, Saccharomyces ellipsoideus, Saccharomyces fermentati, Saccharomyces florentinus, Saccharomyces fragilis, Saccharomyces heterogenicus, Saccharomyces hienipiensis, Saccharomyces inusitatus, Saccharomyces italicus, Saccharomyces kluyveri, Saccharomyces krusei, Saccharomyces lactis, Saccharomyces marxianus, Saccharomyces microellipsoides, Saccharomyces montanus, Saccharomyces norbensis, Saccharomyces oleaceus, Saccharomyces paradoxus, Saccharomyces pastorianus, Saccharomyces pretoriensis, Saccharomyces rosei, Saccharomyces rouxii, Saccharomyces uvarum, Saccharomycodes ludwigii, Yarrowia lipolytica, Schizosacharomycetaceae such as the genera Schizosaccharomyces e.g. the species *Schizosaccharomyces japonicus var. japonicus, Schizosaccharomyces japonicus var. versatilis, Schizosaccharomyces malidevorans, Schizosaccharomyces octosporus, Schizosaccharomyces pombe var. malidevorans, Schizosaccharomyces pombe var. pombe,* Thraustochytriaceae such as the genera Althornia, Aplanochytrium, Japonochytrium, Schizochytrium, Thraustochytrium e.g. the species *Schizochytrium aggregatum, Schizochytrium limacinum, Schizochytrium mangrovei, Schizochytrium minutum, Schizochytrium octosporum, Thraustochytrium aggregatum, Thraustochytrium amoeboideum, Thraustochytrium antacticum, Thraustochytrium arudimentale, Thraustochytrium aureum, Thraustochytrium benthicola, Thraustochytrium globosum, Thraustochytrium indicum, Thraustochytrium kerguelense, Thraustochytrium kinnei, Thraustochytrium motivum, Thraustochytrium multirudimentale, Thraustochytrium pachydermum, Thraustochytrium proliferum, Thraustochytrium roseum, Thraustochytrium rossii, Thraustochytrium striatum* or *Thraustochytrium visurgense.*

Further advantageous microorganisms are, for example, bacteria selected from the group of the families Bacillaceae, Enterobacteriacae or Rhizobiaceae.

Examples which may be mentioned are the following microorganisms selected from the group consisting of: Bacillaceae, such as the genus Bacillus, for example the genera and species *Bacillus acidocaldarius, Bacillus acidoterrestris, Bacillus alcalophilus, Bacillus amyloliquefaciens, Bacillus amylolyticus, Bacillus brevis, Bacillus cereus, Bacillus circulans, Bacillus coagulans, Bacillus sphaericus subsp. fusiformis, Bacillus galactophilus, Bacillus globisporus, Bacillus globisporus subsp. marinus, Bacillus halophilus, Bacillus lentimorbus, Bacillus lentus, Bacillus licheniformis, Bacillus megaterium, Bacillus polymyxa, Bacillus psychrosaccharolyticus, Bacillus pumilus, Bacillus sphaericus, Bacillus subtilis subsp. spizizenii, Bacillus subtilis subsp. subtilis* or *Bacillus thuringiensis;* Enterobacteriacae such as the genera Citrobacter, Edwardsiella, Enterobacter, Erwinia, Escherichia, Klebsiella, Salmonella or Serratia, for example the genera and species *Citrobacter amalonaticus, Citrobacter diversus, Citrobacter freundii, Citrobacter genomospecies, Citrobacter gillenii, Citrobacter intermedium, Citrobacter koseri, Citrobacter murliniae, Citrobacter sp., Edwardsiella hoshinae, Edwardsiella ictaluri, Edwardsiella tarda, Erwinia alni, Erwinia amylovora, Erwinia ananatis, Erwinia aphidicola, Erwinia billingiae, Erwinia cacticida, Erwinia cancerogena, Erwinia carnegieana, Erwinia carotovora* subsp. *atroseptica, Erwinia carotovora* subsp. *betavasculorum, Erwinia carotovora* subsp. *odorifera, Erwinia*

carotovora subsp. *wasabiae, Erwinia chrysanthemi, Erwinia cypripedii, Erwinia dissolvens, Erwinia herbicola, Erwinia mallotivora, Erwinia milletiae, Erwinia nigrifluens, Erwinia nimipressuralis, Erwinia persicina, Erwinia psidii, Erwinia pyrifoliae, Erwinia quercina, Erwinia rhapontici, Erwinia rubrifaciens, Erwinia salicis, Erwinia stewartii, Erwinia tracheiphila, Erwinia uredovora, Escherichia adecarboxylata, Escherichia anindolica, Escherichia aurescens, Escherichia blattae, Escherichia coli, Escherichia coli var. communior, Escherichia coli-mutabile, Escherichia fergusonii, Escherichia hermannii, Escherichia sp., Escherichia vulneris, Klebsiella aerogenes, Klebsiella edwardsii subsp. atlantae, Klebsiella ornithinolytica, Klebsiella oxytoca, Klebsiella planticola, Klebsiella pneumoniae, Klebsiella pneumoniae subsp. pneumoniae, Klebsiella sp., Klebsiella terrigena, Klebsiella trevisanii, Salmonella abony, Salmonella arizonae, Salmonella bongori, Salmonella choleraesuis subsp. arizonae, Salmonella choleraesuis subsp. bongori, Salmonella choleraesuis subsp. cholereasuis, Salmonella choleraesuis subsp. diarizonae, Salmonella choleraesuis subsp. houtenae, Salmonella choleraesuis subsp. indica, Salmonella choleraesuis subsp. salamae, Salmonella daressalaam, Salmonella enterica subsp. houtenae, Salmonella enterica subsp. salamae, Salmonella enteritidis, Salmonella gallinarum, Salmonella heidelberg, Salmonella panama, Salmonella senftenberg, Salmonella typhimurium, Serratia entomophila, Serratia ficaria, Serratia fonticola, Serratia grimesii, Serratia liquefaciens, Serratia marcescens, Serratia marcescens subsp. marcescens, Serratia marinorubra, Serratia odorifera, Serratia plymouthensis, Serratia plymuthica, Serratia proteamaculans, Serratia proteamaculans subsp. quinovora, Serratia quinivorans* or *Serratia rubidaea;* Rhizobiaceae, such as the genera Agrobacterium, Carbophilus, Chelatobacter, Ensifer, Rhizobium, Sinorhizobium, for example the genera and species *Agrobacterium atlanticum, Agrobacterium ferrugineum, Agrobacterium gelatinovorum, Agrobacterium larrymoorei, Agrobacterium meteori, Agrobacterium radiobacter, Agrobacterium rhizogenes, Agrobacterium rubi, Agrobacterium stellulatum, Agrobacterium tumefaciens, Agrobacterium vitis, Carbophilus carboxidus, Chelatobacter heintzii, Ensifer adhaerens, Ensifer arboris, Ensifer fredii, Ensifer kostiensis, Ensifer kummerowiae, Ensifer medicae, Ensifer meliloti, Ensifer saheli, Ensifer terangae, Ensifer xinjiangensis, Rhizobium ciceri Rhizobium etli, Rhizobium fredii, Rhizobium galegae, Rhizobium gallicum, Rhizobium giardinii, Rhizobium hainanense, Rhizobium huakuii, Rhizobium huautlense, Rhizobium indigoferae, Rhizobium japonicum, Rhizobium leguminosarum, Rhizobium loessense, Rhizobium loti, Rhizobium lupini, Rhizobium mediterraneum, Rhizobium meliloti, Rhizobium mongolense, Rhizobium phaseoli, Rhizobium radiobacter, Rhizobium rhizogenes, Rhizobium rubi, Rhizobium sullae, Rhizobium tianshanense, Rhizobium trifolii, Rhizobium tropici, Rhizobium undicola, Rhizobium vitis, Sinorhizobium adhaerens, Sinorhizobium arboris, Sinorhizobium fredii, Sinorhizobium kostiense, Sinorhizobium kummerowiae, Sinorhizobium medicae, Sinorhizobium meliloti, Sinorhizobium morelense, Sinorhizobium saheli* or *Sinorhizobium xinjiangense.*

Further examples of advantageous microorganisms for the process according to the invention are protists or diatoms selected from the group of the families Dinophyceae,

Turaniellidae or Oxytrichidae, such as the genera and species: *Crypthecodinium cohnii, Phaeodactylum tricornutum, Stylonychia mytilus, Stylonychia pustulata, Stylonychia putrina, Stylonychia notophora, Stylonychia sp.,* Colpidium campylum or Colpidium sp.

Those which are advantageously applied in the process according to the invention are transgenic organisms such as fungi, such as mortierella or thraustrochytrium, yeasts such as Saccharomyces or Schizosaccharomyces, mosses such as Physcomitrella or Ceratodon, nonhuman animals such as Caenorhabditis, algae such as Nephroselmis, Pseudoscourfielda, Prasinococcus, Scherffelia, Tetraselmis, Mantoniella, Ostreococcus, Crypthecodinium or Phaeodactylum or plants such as dicotyledonous or monocotyledonous plants. Organisms which are especially advantageously used in the process according to the invention are organisms which belong to the oil-producing organisms, that is to say which are used for the production of oil, such as fungi, such as Mortierella or Thraustochytrium, algae such as Nephroselmis, Pseudoscourfielda, Prasinococcus, Scherffelia, Tetraselmis, Mantoniella, Ostreococcus, Crypthecodinium, Phaeodactylum, or plants, in particular plants, preferably oilseed or oil crop plants which comprise large amounts of lipid compounds, such as peanut, oilseed rape, canola, sunflower, safflower (Carthamus tinctoria), poppy, mustard, hemp, castor-oil plant, olive, sesame, Calendula, Punica, evening primrose, verbascum, thistle, wild roses, hazelnut, almond, macadamia, avocado, bay, pumpkin/squash, linseed, soybean, pistachios, borage, trees (oil palm, coconut or walnut) or arable crops such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa or bushy plants (coffee, cacao, tea), Salix species, and perennial grasses and fodder crops. Preferred plants according to the invention are oil crop plants such as peanut, oilseed rape, canola, sunflower, safflower, poppy, mustard, hemp, castor-oil plant, olive, Calendula, Punica, evening primrose, pumpkin/squash, linseed, soybean, borage, trees (oil palm, coconut). Especially preferred are plants which are high in C18:2- and/or C18:3-fatty acids, such as sunflower, safflower, tobacco, verbascum, sesame, cotton, pumpkin/squash, poppy, evening primrose, walnut, linseed, hemp or thistle. Very especially preferred plants are plants such as safflower, sunflower, poppy, evening primrose, walnut, linseed or hemp.

It is therefore advantageous for the above-described method according to the invention additionally to introduce, into the organism, further nucleic acids which encode enzymes of the fatty acid or lipid metabolism, in addition to the nucleic acids introduced in process step (a) to (d) and to the optionally introduced nucleic acid sequences which encode the ω3-desaturases.

In principle, all genes of the fatty acid or lipid metabolism can be used in the process for the production of polyunsaturated fatty acids, advantageously in combination with the Δ5-elongase(s), Δ6-elongase(s) and/or ω3-desaturases [for the purposes of the present invention, the plural is understood as comprising the singular and vice versa]. Genes of the fatty acid or lipid metabolism selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP

thioesterase(s), fatty acid acyl transferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously used in combination with the Δ5-elongase, Δ6-elongase and/or ω3-desaturase. Genes selected from the group of the Δ4-desaturases, Δ5-desaturases, Δ6-desaturases, Δ8-desaturases, Δ9-desaturases, Δ12-desaturases, Δ6-elongases or Δ9-elongases are especially preferably used in combination with the above genes for the Δ5-elongase, Δ6-elongase and/or ω3-desaturase, it being possible to use individual genes or a plurality of genes in combination.

In comparison with the human elongases or elongases from nonhuman animals such as those from Oncorhynchus, Xenopus or Ciona, the Δ5-elongases according to the invention have the advantageous property that they do not elongate C_{22}-fatty acids to the corresponding C_{24}-fatty acids. Furthermore, they advantageously do not convert fatty acids with a double bond in Δ6-position, as are converted by the human elongases or the elongases from nonhuman animals. Especially advantageous Δ5-elongases preferentially only convert unsaturated C_{20}-fatty acids.These advantageous Δ5-elongases have some putative transmembrane helices (5-7). Advantageously, only C_{20}-fatty acids with one double bond in Δ5-position are converted, with ω3-C_{20}-fatty acids being preferred (EPA). In a preferred embodiment of the invention, they furthermore have the property that they advantageously have no, or only relatively little, Δ6-elongase activity, in addition to the Δ5-elongase activity. In contrast, the human elongases or elongases from nonhuman animals have approximately the same activity on fatty acids with a Δ6- or Δ5-double bond. These advantageous elongases are referred to as what are known as monofunctional elongases. The human elongases or the elongases from nonhuman animals, in contrast, are referred to as multifunctional elongases which, in addition to the abovementioned substrates, also convert monounsaturated C_{16}- and C_{18}-fatty acids, for example with a Δ9- or Δ11-double bond. In a yeast feeding test in which EPA had been added to the yeasts to act as substrate, the monofunctional elongases advantageously convert at least 15% of the added EPAs into docosapentaenoic acid (DPA, $C22:5^{\Delta 7,10,13,16,19}$), advantageously at least 20% by weight, especially advantageously at least 25% by weight. If γ-linolenic acid (= GLA, $C18:3^{\Delta 6,9,12}$) is added as substrate, this substance is advantageously not elongated at all. $C18:3^{\Delta 5,9,12}$ is likewise not elongated. In another advantageous embodiment, less than 60% by weight, advantageously less than 55% by weight, especially preferably less than 50% by weight, especially advantageously less than 45% by weight, very especially advantageously less than 40% by weight, of the added GLA are converted into dihomo- γ-linolenic acid (= $C20:3^{\Delta 8,11,14}$). In a further, very especially preferred embodiment of the Δ5-elongase activity according to the invention, GLA is not converted.

Figures 27 and 28 show the measured substrate specificities of the different elongases. Figure 27 shows the specifities of the multifunctional elongases of Xenopus laevis

(Fig. 27 A), Ciona intestinalis (Fig. 27 B) and Oncorhynchus mykiss (Fig. 27 C). All of these elongases convert a broad spectrum of substrates. In the method according to the invention, this can give rise to by-products which must be converted by further enzymatic activities. This is why these enzymes are less preferred in the method according to the invention. The preferred monofunctional elongases and their substrate specificity are shown in Figure 28. Figure 28 A shows the specificity of the Ostreococcus tauri Δ5-elongase. This enzyme only converts fatty acids with a double bond in the Δ5-position. Advantageously, only C20-fatty acids are converted. A similarly high substrate specificity is shown by the Thalassiosira pseudonana Δ5-elongase (Fig. 28 C). Both the Ostreococcus tauri Δ6-elongase (Fig. 28 B) and that of Thalassiosira pseudonana (Fig. 28 D) advantageously only convert fatty acids with a double bond in the Δ6-position. Advantageously, only C18-fatty acids are converted. The Δ5-elongases from Arabidopsis thaliana and Euglena gracilis are also distinguished by their specificity.

Advantageous Δ6-elongases according to the invention are likewise distinguished by high specificity, that is to say that C_{18}-fatty acids are elongated by preference. Advantageously, they convert fatty acids with a double bond in the Δ6-position. Especially advantageous Δ6-elongases advantageously convert C_{18}-fatty acids with three or four double bonds in the molecule, which fatty acids must comprise one double bond in the Δ6-position. In a preferred embodiment of the invention, they furthermore have the characteristic that they advantageously have no, or only relatively little, Δ5-elongase activity, besides the Δ6-elongase activity. In contrast, the human elongases or elongases from nonhuman animals have approximately the same activity on fatty acids with a Δ6- or Δ5-double bond. These advantageous elongases are referred to as what are known as monofunctional elongases. As described above, the human elongases or the elongases from nonhuman animals are referred to, in contrast, as multifunctional elongases which, besides the abovementioned substrates, also convert monounsaturated C_{16}- and C_{18}-fatty acids, for example with Δ9- or Δ11-double bond. In a yeast feeding test in which EPA had been added to the yeast to act as substrate, the monofunctional elongases advantageously convert at least 10% by weight of the added α-linolenic acid (= ALA, $C18:3^{\Delta 9,12,15}$) or at least 40% by weight of the added γ-linolenic acid (= GLA, $C18:3^{\Delta 6,9,12}$), advantageously at least 20% by weight or 50% by weight, especially advantageously at least 25% by weight or 60% by weight. It is especially advantageous that $C18:4^{\Delta 6,9,12,15}$ (stearidonic acid) is also elongated. In this context, SDA is converted to at least 40% by weight, advantageously to at least 50% by weight, especially advantageously to at least 60% by weight, very especially advantageously to at least 70% by weight. Especially advantageous Δ6-elongases show no or only very little activity (conversion rate less than 0.1% by weight) toward the following substrates: $C18:1^{\Delta 6}$, $C18:1^{\Delta 9}$, $C18:1^{\Delta 11}$, $C20:2^{\Delta 11,14}$, $C20:3^{\Delta 11,14,17}$, $C20:3^{\Delta 8,11,14}$, $C20:4^{\Delta 5,8,11,14}$, $C20:5^{\Delta 5,8,11,14,17}$ or $C22:4^{\Delta 7,10,13,16}$.

Figures 29 and 30 and table 18 show the measured substrate specificities of the various elongases.

In contrast with the known ω3-desaturase, the ω3-desaturase according to the invention has the advantageous characteristic that it is capable of desaturating a broad spectrum of ω6-fatty acids; C_{20}- and C_{22}-fatty acids such as $C_{20:2}$-, $C_{20:3}$-, $C_{20:4}$-, $C_{22:4}$- or $C_{22:5}$-fatty acids are desaturated by preference. However, the shorter C_{18}-fatty acids such as $C_{18:2}$- or $C_{18:3}$-fatty acids are also advantageously desaturated. Owing to these characteristics of the ω3-desaturase, it is advantageously possible to shift the fatty acid spectrum within an organism, advantageously within a plant or a fungus, from the ω6-fatty acids toward the ω3-fatty acids. Preferably, the ω3-desaturase according to the invention desaturates C_{20}-fatty acids. Within the organism, these fatty acids from the existing fatty acid pool are converted to at least 10%, 15%, 20%, 25% or 30% into the corresponding ω3-fatty acids. The activity of the enzyme ω3-desaturase toward the C_{18}-fatty acids is lower by a factor of10, i.e. only approximately 1.5 to 3% of the fatty acids present in the fatty acid pool are converted into the corresponding ω3-fatty acids. Preferred substrate of the ω3-desaturase according to the invention are the ω6-fatty acids which are bound in phospholipids. Figure 19 demonstrates clearly with reference to the desaturation of dihomo-γ-linolenic acid [$C_{20:4}^{\Delta 8,11,14}$], that, during the desaturation process, the ω3-desaturase advantageously does not distinguish between fatty acids which are bound at the sn1 position or at the sn2 position. Both fatty acids bound at the sn1 position and fatty acids bound at the sn2 position in the phospholipids are desaturated. Furthermore, it is advantageous that the ω3-desaturase converts a broad range of phospholipids such as phosphatidylcholine (= PC), phosphatidylinositol (= PIS) or phosphatidylethanolamine (= PE). Finally, desaturation products can also be found in the neutral lipids (= NL), that is to say in the triglycerides.

In comparison with the known Δ4-desaturases, Δ5-desaturases and Δ6-desaturases, the advantage of the Δ4-desaturases, Δ5-desaturases and Δ6-desaturases according to the invention is that they can convert fatty acids which are bound to phospholipids or CoA-fatty acid esters, advantageously CoA-fatty acid esters.

The Δ12-desaturases used in the process according to the invention advantageously convert oleic acid ($C18:1^{\Delta 9}$) into linoleic acid ($C18:2^{\Delta 9,12}$) or $C18:2^{\Delta 6,9}$ into $C18:3^{\Delta 6,9,12}$ (= GLA). The Δ12-desaturases used advantageously convert fatty acids which are bound to phospholipids or CoA-fatty acid esters, advantageously those which are bound to CoA-fatty acid esters.

Owing to the enzymatic activity of the nucleic acids used in the process according to the invention which encode polypeptides with Δ5-elongase, Δ6-elongase and/or ω3-desaturase activity, advantageously in combination with nucleic acid sequences which encode polypeptides of the fatty acid or lipid metabolism, such as additional polypeptides with Δ4-, Δ5-, Δ6-, Δ8-, Δ12-desaturase or Δ5-, Δ6- or Δ9-elongase activity, a wide range of polyunsaturated fatty acids can be produced in the process according to the invention. Depending on the choice of the organisms, such as the advantageous plants, used for the process according to the invention, mixtures of the various polyunsaturated fatty acids or individual polyunsaturated fatty acids, such as EPA or ARA, can be produced in free or bound form. Depending on the prevailing fatty

acid composition in the starting plant (C18:2- or C18:3-fatty acids), fatty acids which are derived from C18:2-fatty acids, such as GLA, DGLA or ARA, or fatty acids which are derived from C18:3-fatty acids, such as SDA, ETA or EPA, are thus obtained. If only linoleic acid (= LA, C18:$2^{\Delta9,12}$) is present as unsaturated fatty acid in the plant used for the process, the process can only afford GLA, DGLA and ARA as products, all of which can be present as free fatty acids or in bound form. If only α-linolenic acid (= ALA, C18:$3^{\Delta9,12,15}$) is present as unsaturated fatty acid in the plant used for the process, as is the case, for example, in linseed, the process can only afford SDA, ETA or EPA and/or DHA as products, all of which can be present as free fatty acids or in bound form, as described above. Owing to the modification of the activity of the enzyme Δ5-elongase advantageously in combination with Δ4-, Δ5-, Δ6-, Δ12-desaturase, and/or Δ6-elongase, or Δ4-, Δ5-, Δ8-, Δ12-desaturase, and/or Δ9-elongase which play a role in the synthesis, it is possible to produce, in a targeted fashion, only individual products in the abovementioned organisms, advantageously in the abovementioned plants. Owing to the activity of Δ6-desaturase and Δ6-elongase, for example, GLA and DGLA, or SDA and ETA, are formed, depending on the starting plant and unsaturated fatty acid. DGLA or ETA or mixtures of these are preferably formed. If Δ5-desaturase, Δ5-elongase and Δ4-desaturase are additionally introduced into the organisms, advantageously into the plant, ARA, EPA and/or DHA are additionally formed. This also applies to organisms into which the Δ8-desaturase and Δ9-elongase had previously been introduced. Advantageously, only ARA, EPA or DHA or mixtures of these are synthesized, depending on the fatty acid present in the organism, or in the plant, which acts as starting substance for the synthesis. Since biosynthetic cascades are involved, the end products in question are not present in pure form in the organisms. Small amounts of the precursor compounds are always additionally present in the end product. These small amounts amount to less than 20% by weight, advantageously less than 15% by weight, especially advantageously less than 10% by weight, most advantageously less than 5, 4, 3, 2 or 1% by weight, based on the end products DGLA, ETA or their mixtures, or ARA, EPA, DHA or their mixtures, advantageously EPA or DHA or their mixtures.

The protein encoded by the nucleic acid according to the invention demonstrates high specificity for the two precursors C18:$4^{\Delta6,9,12,15}$- and C20:$5^{\Delta5,8,11,14,17}$-fatty acids for the synthesis of DHA (precursors and synthesis of DHA, see figure 1). Thus, the protein encoded by SEQ NO: 53 has specificity for Δ6- and Δ5-fatty acids with additionally one ω3-double bond (figure 2). Δ5-elongase has ketoacyl-CoA synthase activity which advantageously elongates fatty acid residues of acyl-CoA esters by 2 carbon atoms.

With the aid of the Δ5-elongase genes, the Phaeodacylum Δ5-desaturase and the Euglena Δ4-desaturase, it was possible to demonstrate the synthesis of DHA in yeast (Saccharomyces cerevisiae) (figure 3).

In addition to the production directly in the organism, of the starting fatty acids for the Δ5-elongase, Δ6-elongase and/or ω3-desaturase of the invention, the fatty acids can

also be fed externally. The production in the organism is preferred for reasons of economy. Preferred substrates of ω3-desaturase are linoleic acid (C18:$2^{\Delta9,12}$), γ-linolenic acid (C18:$3^{\Delta6,9,12}$), eicosadienoic acid (C20:$2^{\Delta11,14}$), dihomo-γ-linolenic acid (C20:$3^{\Delta8,11,14}$), arachidonic acid (C20:$4^{\Delta5,8,11,14}$), docosatetraenoic acid (C22:$4^{\Delta7,10,13,16}$) and docosapentaenoic acid (C22:$5^{\Delta4,7,10,13,15}$).

To increase the yield in the above-described process for the production of oils and/or triglycerides with an advantageously elevated content of polyunsaturated fatty acids, it is advantageous to increase the amount of starting product for the synthesis of fatty acids; this can be achieved for example by introducing, into the organism, a nucleic acid which encodes a polypeptide with Δ12-desaturase. This is particularly advantageous in oil-producing organisms such as those from the family of the Brassicaceae, such as the genus Brassica, for example oilseed rape; the family of the Elaeagnaceae, such as the genus Elaeagnus, for example the genus and species *Olea europaea*, or the family Fabaceae, such as the genus Glycine, for example the genus and species *Glycine max,* which are high in oleic acid. Since these organisms are only low in linoleic acid (Mikoklajczak et al., Journal of the American Oil Chemical Society, 38, 1961, 678 - 681), the use of the abovementioned Δ12-desaturases for producing the starting material linoleic acid is advantageous.

Nucleic acids used in the process according to the invention are advantageously derived from plants such as algae, for example algae of the family of the Prasinophyceae such as the genera Heteromastix, Mammella, Mantoniella, Micromonas, Nephroselmis, Ostreococcus, Prasinocladus, Prasinococcus, Pseudoscourfielda, Pycnococcus, Pyramimonas, Scherffelia or Tetraselmis such as the genera and species Heteromastix longifillis, Mamiella gilva, Mantoniella squamata, Micromonas pusilla, Nephroselmis olivacea, Nephroselmis pyriformis, Nephroselmis rotunda, Ostreococcus tauri, Ostreococcus sp. Prasinocladus ascus, Prasinocladus lubricus, Pycnococcus provasolii, Pyramimonas amylifera, Pyramimonas disomata, Pyramimonas obovata, Pyramimonas orientalis, Pyramimonas parkeae, Pyramimonas spinifera, Pyramimonas sp., Tetraselmis apiculata, Tetraselmis carteriaformis, Tetraselmis chui, Tetraselmis convolutae, Tetraselmis desikacharyi, Tetraselmis gracilis, Tetraselmis hazeni, Tetraselmis impellucida, Tetraselmis inconspicua, Tetraselmis levis, Tetraselmis maculata, Tetraselmis marina, Tetraselmis striata, Tetraselmis subcordiformis, Tetraselmis suecica, Tetraselmis tetrabrachia, Tetraselmis tetrathele, Tetraselmis verrucosa, Tetraselmis verrucosa fo. rubens or Tetraselmis sp. or from algae of the family Euglenaceae such as the genera Ascoglena, Astasia, Colacium, Cyclidiopsis, Euglena, Euglenopsis, Hyalophacus, Khawkinea, Lepocinclis, Phacus, Strombomonas or Trachelomonas, such as the genera and species Euglena acus, Euglena geniculata, Euglena gracilis, Euglena mixocylindracea, Euglena rostrifera, Euglena viridis, Colacium stentorium, Trachelomonas cylindrica or Trachelomonas volvocina. The nucleic acids used are advantageously derived from algae of the genera Euglena, Mantoniella or Ostreococcus.

Further advantageous plants are algae such as Isochrysis or Crypthecodinium,

algae/diatoms such as Thalassiosira or Phaeodactylum, mosses such as Physcomitrella or Ceratodon, or higher plants such as the Primulaceae such as Aleuritia, Calendula stellata, Osteospermum spinescens or Osteospermum hyoseroides, microorganisms such as fungi, such as Aspergillus, Thraustochytrium, Phytophthora, Entomophthora, Mucor or Mortierella, bacteria such as Shewanella, yeasts or animals such as nematodes such as Caenorhabditis, insects, frogs, abalone, or fish. The isolated nucleic acid sequences according to the invention are advantageously derived from an animal of the order of the vertebrates. Preferably, the nucleic acid sequences are derived from the classes of the Vertebrata; Euteleostomi, Actinopterygii; Neopterygii; Teleostei; Euteleostei, Protacanthopterygii, Salmoniformes; Salmonidae or Oncorhynchus or Vertebrata, Amphibia, Anura, Pipidae, Xenopus or Evertebrata such as Protochordata, Tunicata, Holothuroidea, Cionidae such as Amaroucium constellatum, Botryllus schlosseri, Ciona intestinalis, Molgula citrina, Molgula manhattensis, Perophora viridis or Styela partita. The nucleic acids are especially advantageously derived from fungi, animals, or from plants such as algae or mosses, preferably from the order of the Salmoniformes, such as the family of the Salmonidae, such as the genus Salmo, for example from the genera and species Oncorhynchus mykiss, Trutta trutta or Salmo trutta fario, from algae, such as the genera Mantoniella or Ostreococcus, or from the diatoms such as the genera Thalassiosira or Phaeodactylum or from algae such as Crypthecodinium.

The process according to the invention advantageously the abovementioned nucleic acid sequences or their derivatives or homologues which encode polypeptides which retain the enzymatic activity of the proteins encoded by nucleic acid sequences. These sequences, individually or in combination with the nucleic acid sequences which encode Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ5-elongase, Δ6-elongase and/or ω3-desaturase, are cloned into expression constructs and used for the introduction into, and expression in, organisms. Owing to their construction, these expression constructs make possible an advantageous optimal synthesis of the polyunsaturated fatty acids produced in the process according to the invention.

In a preferred embodiment, the process furthermore comprises the step of obtaining a cell or an intact organism which comprises the nucleic acid sequences used in the process, where the cell and/or the organism is transformed with a nucleic acid sequence according to the invention which encodes the Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ5-elongase, Δ6-elongase and/or ω3-desaturase, a gene construct or a vector as described above, alone or in combination with further nucleic acid sequences which encode proteins of the fatty acid or lipid metabolism. In a further preferred embodiment, this process furthermore comprises the step of obtaining the oils, lipids or free fatty acids from the organism or from the culture. The culture can, for example, take the form of a fermentation culture, for example in the case of the cultivation of microorganisms, such as, for example, Mortierella, Thalassiosira, Mantoniella, Ostreococcus, Saccharomyces or Thraustochytrium, or a greenhouse- or field-grown culture of a plant. The cell or the organism produced thus is advantageously a cell of an oil-producing organism, such as

an oil crop, such as, for example, peanut, oilseed rape, canola, linseed, hemp, peanut, soybean, safflower, hemp, sunflowers or borage.

In the case of plant cells, plant tissue or plant organs, “growing” is understood as meaning, for example, the cultivation on or in a nutrient medium, or of the intact plant on or in a substrate, for example in a hydroponic culture, potting compost or on arable land.

For the purposes of the invention, "transgenic" or "recombinant" means with regard to, for example, a nucleic acid sequence, an expression cassette (= gene construct) or a vector comprising the nucleic acid sequence or an organism transformed with the nucleic acid sequences, expression cassettes or vectors according to the invention, all those constructions brought about by recombinant methods in which either

a) the nucleic acid sequence according to the invention, or

b) a genetic control sequence which is operably linked with the nucleic acid sequence according to the invention, for example a promoter, or

c) a) and b)

are not located in their natural genetic environment or have been modified by recombinant methods, it being possible for the modification to take the form of, for example, a substitution, addition, deletion, inversion or insertion of one or more nucleotide residues. The natural genetic environment is understood as meaning the natural genomic or chromosomal locus in the original organism or the presence in a genomic library. In the case of a genomic library, the natural genetic environment of the nucleic acid sequence is preferably retained, at least in part. The environment flanks the nucleic acid sequence at least on one side and has a sequence length of at least 50 bp, preferably at least 500 bp, especially preferably at least 1000 bp, most preferably at least 5000 bp. A naturally occurring expression cassette – for example the naturally occurring combination of the natural promoter of the nucleic acid sequences with the corresponding Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, ω3-desaturase, Δ9-elongase, Δ6-elongase and/or Δ5-elongase genes – becomes a transgenic expression cassette when this expression cassette is modified by non-natural, synthetic ("artificial") methods such as, for example, mutagenic treatment. Suitable methods are described, for example, in US 5,565,350 or WO 00/15815.

A transgenic organism or transgenic plant for the purposes of the invention is therefore understood as meaning, as above, that the nucleic acids used in the process are not at their natural locus in the genome of an organism, it being possible for the nucleic acids to be expressed homologously or heterologously. However, as mentioned, transgenic also means that, while the nucleic acids according to the invention are at their natural position in the genome of an organism, the sequence has been modified with regard to the natural sequence, and/or that the regulatory sequences of the natural sequences

have been modified. Transgenic is preferably understood as meaning the expression of the nucleic acids according to the invention at an unnatural locus in the genome, i.e. homologous or, preferably, heterologous expression of the nucleic acids takes place. Preferred transgenic organisms are fungi such as Mortierella or Phytophtora, mosses such as Physcomitrella, algae such as Mantoniella, Euglena, Crypthecodinium or Ostreococcus, diatoms such as Thalassiosira or Phaeodactylum, or plants such as the oil crops.

Organisms or host organisms for the nucleic acids, the expression cassette or the vector used in the process according to the invention are, in principle, advantageously all organisms which are capable of synthesizing fatty acids, specifically unsaturated fatty acids, and/or which are suitable for the expression of recombinant genes. Examples which may be mentioned are plants such as Arabidopsis, Asteraceae such as Calendula or crop plants such as soybean, peanut, castor-oil plant, sunflower, maize, cotton, flax, oilseed rape, coconut, oil palm, safflower (Carthamus tinctorius) or cacao bean, microorganisms, such as fungi, for example the genus Mortierella, Thraustochytrium, Saprolegnia, Phytophtora or Pythium, bacteria, such as the genus Escherichia or Shewanella, yeasts, such as the genus Saccharomyces, cyanobacteria, ciliates, algae such as Mantoniella, Euglena, Thalassiosira or Ostreococcus, or protozoans such as dinoflagellates, such as Crypthecodinium. Preferred organisms are those which are naturally capable of synthesizing substantial amounts of oil, such as fungi, such as Mortierella alpina, Pythium insidiosum, Phytophtora infestans, or plants such as soybean, oilseed rape, coconut, oil palm, safflower, flax, hemp, castor-oil plant, Calendula, peanut, cacao bean or sunflower, or yeasts such as Saccharomyces cerevisiae with soybean, flax, oilseed rape, safflower, sunflower, Calendula, Mortierella or Saccharomyces cerevisiae being especially preferred. In principle, host organisms are, in addition to the abovementioned transgenic organisms, also transgenic animals, advantageously nonhuman animals, for example C. elegans, Ciona intestinalis or Xenopus laevis.

Further utilizable host cells are detailed in: Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990).

Expression strains which can be used, for example those with a lower protease activity, are described in: Gottesman, S., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 119-128.

These include plant cells and certain tissues, organs and parts of plants in all their phenotypic forms such as anthers, fibers, root hairs, stalks, embryos, calli, cotelydons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant.

Transgenic plants which comprise the polyunsaturated fatty acids synthesized in the process according to the invention can advantageously be marketed directly without

there being any need for the oils, lipids or fatty acids synthesized to be isolated. Plants for the process according to the invention are listed as meaning intact plants and all plant parts, plant organs or plant parts such as leaf, stem, seeds, root, tubers, anthers, fibers, root hairs, stalks, embryos, calli, cotelydons, petioles, harvested material, plant tissue, reproductive tissue and cell cultures which are derived from the actual transgenic plant and/or can be used for bringing about the transgenic plant. In this context, the seed comprises all parts of the seed such as the seed coats, epidermal cells, seed cells, endosperm or embryonic tissue. However, the compounds produced in the process according to the invention can also be isolated from the organisms, advantageously plants, in the form of their oils, fats, lipids and/or free fatty acids. Polyunsaturated fatty acids produced by this process can be obtained by harvesting the organisms, either from the crop in which they grow, or from the field. This can be done via pressing or extraction of the plant parts, preferably the plant seeds. In this context, the oils, fats, lipids and/or free fatty acids can be obtained by what is known as cold-beating or cold-pressing without applying heat. To allow for greater ease of disruption of the plant parts, specifically the seeds, they are previously comminuted, steamed or roasted. The seeds which have been pretreated in this manner can subsequently be pressed or extracted with solvents such as warm hexane. The solvent is subsequently removed. In the case of microorganisms, the latter are, after harvesting, for example extracted directly without further processing steps or else, after disruption, extracted via various methods with which the skilled worker is familiar. In this manner, more than 96% of the compounds produced in the process can be isolated. Thereafter, the resulting products are processed further, i.e. refined. In this process, substances such as the plant mucilages and suspended matter are first removed. What is known as desliming can be effected enzymatically or, for example, chemico-physically by addition of acid such as phosphoric acid. Thereafter, the free fatty acids are removed by treatment with a base, for example sodium hydroxide solution. The resulting product is washed thoroughly with water to remove the alkali remaining in the product and then dried. To remove the pigment remaining in the product, the products are subjected to bleaching, for example using filler's earth or active charcoal. At the end, the product is deodorized, for example using steam.

The PUFAs or LCPUFAs produced by this process are advantageously C_{18}-, D_{20}- or C_{22}-fatty acid molecules, advantageously C_{20}- or C_{22}-fatty acid molecules, with at least two double bonds in the fatty acid molecule, preferably three, four, five or six double bonds. These C_{18}-, C_{20}- or C_{22}-fatty acid molecules can be isolated from the organism in the form of an oil, a lipid or a free fatty acid. Suitable organisms are, for example, those mentioned above. Preferred organisms are transgenic plants.

One embodiment of the invention is therefore oils, lipids or fatty acids or fractions thereof which have been produced by the above-described process, especially preferably oil, lipid or a fatty acid composition comprising PUFAs and being derived from transgenic plants.

As described above, these oils, lipids or fatty acids advantageously comprise 6 to 15%

of palmitic acid, 1 to 6% of stearic acid, 7-85% of oleic acid, 0.5 to 8% of vaccenic acid, 0.1 to 1% of arachic acid, 7 to 25% of saturated fatty acids, 8 to 85% of monounsaturated fatty acids and 60 to 85% of polyunsaturated fatty acids, in each case based on 100% and on the total fatty acid content of the organisms. Advantageous polyunsaturated fatty acids which are present in the fatty acid esters or fatty acid mixtures are preferably at least 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 or 1% of arachidonic acid, based on the total fatty acid content. Moreover, the fatty acid esters or fatty acid mixtures which have been produced by the process of the invention advantageously comprise fatty acids selected from the group of the fatty acids erucic acid (13-docosaenoic acid), sterculic acid (9,10-methyleneoctadec-9-enoic acid), malvalic acid (8,9-methyleneheptadec-8-enoic acid), chaulmoogric acid (cyclo-pentenedodecanoic acid), furan fatty acid (9,12-epoxyoctadeca-9,11-dienoic acid), vernolic acid (9,10-epoxyoctadec-12-enoic acid), tariric acid (6-octadecynoic acid), 6-nonadecynoic acid, santalbic acid (t11-octadecen-9-ynoic acid), 6,9-octadecenynoic acid, pyrulic acid (t10-heptadecen-8-ynoic acid), crepenyninic acid (9-octadecen-12-ynoic acid), 13,14-dihydrooropheic acid, octadecen-13-ene-9,11-diynoic acid, petroselenic acid (cis-6-octadecenoic acid), 9c,12t-octadecadienoic acid, calendulic acid (8t10t12c-octadecatrienoic acid), catalpic acid (9t11t13c-octadecatrienoic acid), eleostearic acid (9c11t13t-octadecatrienoic acid), jacaric acid (8c10t12c-octadeca-trienoic acid), punicic acid (9c11t13c-octadecatrienoic acid), parinaric acid (9c11t13t15c-octadecatetraenoic acid), pinolenic acid (all-cis-5,9,12-octadecatrienoic acid), laballenic acid (5,6-octadecadienallenic acid), ricinoleic acid (12-hydroxyoleic acid) and/or coriolic acid (13-hydroxy-9c,11t-octadecadienoic acid). The abovementioned fatty acids are, as a rule, advantageously only found in traces in the fatty acid esters or fatty acid mixtures produced by the process according to the invention, that is to say that, based on the total fatty acids, they occur to less than 30%, preferably to less than 25%, 24%, 23%, 22% or 21%, especially preferably to less than 20%, 15%, 10%, 9%, 8%, 7%, 6% or 5%, very especially preferably to less than 4%, 3%, 2% or 1%. In a further preferred form of the invention, these abovementioned fatty acids occur to less than 0.9%, 0.8%, 0.7%, 0.6% or 0.5%, especially preferably to less than 0.4%, 0.3%, 0.2%, 0.1%, based on the total fatty acids. The fatty acid esters or fatty acid mixtures produced by the process according to the invention advantageously comprise less than 0.1%, based on the total fatty acids, and/or no butyric acid, no cholesterol, no clupanodonic acid (= docosapentaenoic acid, $C22:5^{\Delta 4,8,12,15,21}$) and no nisinic acid (tetracosahexaenoic acid, $C23:6^{\Delta 3,8,12,15,18,21}$).

The oils, lipids or fatty acids according to the invention advantageously comprise at least 0.5%, 1%, 2%, 3%, 4% or 5%, advantageously at least 6%, 7%, 8%, 9% or 10%, especially advantageously at least 11%, 12%, 13%, 14% or 15% of ARA or at least 0.5%, 1%, 2%, 3%, 4% or 5%, advantageously at least 6% or 7%, especially advantageously at least 8%, 9% or 10% of EPA and/or DHA, based on the total fatty acid content of the production organism, advantageously of a plant, especially preferably of an oil crop plant such as soybean, oilseed rape, coconut, oil palm, safflower, flax, hemp, castor-oil plant, Calendula, peanut, cacao bean, sunflower, or the

abovementioned further mono- or dicotyledonous oil crop plants.

A further embodiment according to the invention is the use of the oil, lipid, the fatty acids and/or the fatty acid composition in feedstuffs, foodstuffs, cosmetics or pharmaceuticals. The oils, lipids, fatty acids or fatty acid mixtures according to the invention can be used in the manner with which the skilled worker is familiar for mixing with other oils, lipids, fatty acids or fatty acid mixtures of animal origin, such as, for example, fish oils. These oils, lipids, fatty acids or fatty acid mixtures, which are composed of vegetable and animal constituents, may also be used for the preparation of feedstuffs, foodstuffs, cosmetics or pharmacologicals.

The term "oil", "lipid" or "fat" is understood as meaning a fatty acid mixture comprising unsaturated, saturated, preferably esterified, fatty acid(s). The oil, lipid or fat is preferably high in polyunsaturated free or, advantageously, esterified fatty acid(s), in particular linoleic acid, γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, α-linolenic acid, stearidonic acid, eicosatetraenoic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acid.

The amount of unsaturated esterified fatty acids preferably amounts to approximately 30%, a content of 50% is more preferred, a content of 60%, 70%, 80% or more is even more preferred. For the analysis, the fatty acid content can, for example, be determined by gas chromatography after converting the fatty acids into the methyl esters by transesterification. The oil, lipid or fat can comprise various other saturated or unsaturated fatty acids, for example calendulic acid, palmitic acid, palmitoleic acid, stearic acid, oleic acid and the like. The content of the various fatty acids in the oil or fat can vary, in particular depending on the starting organism.

The polyunsaturated fatty acids with advantageously at least two double bonds which are produced in the process are, as described above, for example sphingolipids, phosphoglycerides, lipids, glycolipids, phospholipids, monoacylglycerol, diacylglycerol, triacylglycerol or other fatty acid esters.
Starting from the polyunsaturated fatty acids with advantageously at least five or six double bonds, which acids have been prepared in the process according to the invention, the polyunsaturated fatty acids which are present can be liberated for example via treatment with alkali, for example aqueous KOH or NaOH, or acid hydrolysis, advantageously in the presence of an alcohol such as methanol or ethanol, or via enzymatic cleavage, and isolated via, for example, phase separation and subsequent acidification via, for example, H_2SO_4. The fatty acids can also be liberated directly without the above-described processing step.

After their introduction into an organism, advantageously a plant cell or plant, the nucleic acids used in the process can either be present on a separate plasmid or, advantageously, integrated into the genome of the host cell. In the case of integration into the genome, integration can be random or else be effected by recombination such that the native gene is replaced by the copy introduced, whereby the production of the

desired compound by the cell is modulated, or by the use of a gene in trans, so that the gene is linked operably with a functional expression unit which comprises at least one sequence which ensures the expression of a gene and at least one sequence which ensures the polyadenylation of a functionally transcribed gene. The nucleic acids are advantageously introduced into the organisms via multiexpression cassettes or constructs for multiparallel expression, advantageously into the plants for the multiparallel seed-specific expression of genes.

Mosses and algae are the only known plant systems which produce substantial amounts of polyunsaturated fatty acids such as arachidonic acid (ARA) and/or eicosapentaenoic acid (EPA) and/or docosahexaenoic acid (DHA). Mosses comprise PUFAs in membrane lipids, while algae, organisms which are related to algae and a few fungi also accumulate substantial amounts of PUFAs in the triacylglycerol fraction. This is why nucleic acid molecules which are isolated from such strains which also accumulate PUFAs in the triacylglycerol fraction are particularly advantageous for the process according to the invention and thus for the modification of the lipid and PUFA production system in a host, in particular plants such as oil crops, for example oilseed rape, canola, linseed, hemp, soybeans, sunflowers and borage. They can therefore be used advantageously in the process according to the invention.

Substrates which are advantageously suitable for the nucleic acids which are used in the process according to the invention and which encode polypeptides with Δ12-desaturase, Δ5-desaturase, Δ4-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-elongase, Δ5-elongase, Δ6-elongase and/or ω3-desaturase activity and/or the further nucleic acids used, such as the nucleic acids which encode polypeptides of the fatty acid or lipid metabolism selected from the group acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl–ACP thioesterase(s),fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl–coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) are advantageously C_{16}-, C_{18}- or C_{20}-fatty acids. The fatty acids converted as substrates in the process are preferably converted in the form of their acyl-CoA esters and/or their phospholipid esters.

To produce the long-chain PUFAs according to the invention, the polyunsaturated C_{18}-fatty acids must first be desaturated by the enzymatic activity of a desaturase and subsequently be elongated by at least two carbon atoms via an elongase. After one elongation cycle, this enzyme activity gives C_{20}-fatty acids and after two elongation cycles C_{22}-fatty acids. The activity of the desaturases and elongases used in the process according to the invention preferably leads to C_{18}-, C_{20}- and/or C_{22}-fatty acids, advantageously with at least two double bonds in the fatty acid molecule, preferably with three, four, five or six double bonds, especially preferably to give C_{20}- and/or C_{22}-fatty acids with at least two double bonds in the fatty acid molecule, preferably with three, four, five or six double bonds, very specially preferably with five or six double

bonds in the molecule. After a first desaturation and the elongation have taken place, further desaturation and elongation steps such as, for example, such a desaturation in the Δ5 and Δ4 position may take place. Products of the process according to the invention which are especially preferred are dihomo-γ-linolenic acid, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid and/or docosahexaenoic acid. The C_{20}-fatty acids with at least two double bonds in the fatty acid can be elongated by the enzymatic activity according to the invention in the form of the free fatty acid or in the form of the esters, such as phospholipids, glycolipids, sphingolipids, phosphoglycerides, monoacylglycerol, diacylglycerol or triacylglycerol.

The preferred biosynthesis site of the fatty acids, oils, lipids or fats in the plants which are advantageously used is, for example, in general the seed or cell strata of the seed, so that seed-specific expression of the nucleic acids used in the process makes sense. However, it is obvious that the biosynthesis of fatty acids, oils or lipids need not be limited to the seed tissue, but can also take place in a tissue-specific manner in all the other parts of the plant, for example in epidermal cells or in the tubers.

If microorganisms such as yeasts, such as Saccharomyces or Schizosaccharomyces, fungi such as Mortierella, Aspergillus, Phytophtora, Entomophthora, Mucor or Thraustochytrium, algae such as Isochrysis, Mantoniella, Euglena, Ostreococcus, Phaeodactylum or Crypthecodinium are used as organisms in the process according to the invention, these organisms are advantageously grown in fermentation cultures.

Owing to the use of the nucleic acids according to the invention which encode a Δ5-elongase, the polyunsaturated fatty acids produced in the process can be increased by at least 5%, preferably by at least 10%, especially preferably by at least 20%, very especially preferably by at least 50% in comparison with the wild types of the organisms which do not comprise the nucleic acids recombinantly.

In principle, the polyunsaturated fatty acids produced by the process according to the invention in the organisms used in the process can be increased in two different ways. Advantageously, the pool of free polyunsaturated fatty acids and/or the content of the esterified polyunsaturated fatty acids produced via the process can be enlarged. Advantageously, the pool of esterified polyunsaturated fatty acids in the transgenic organisms is enlarged by the process according to the invention.

If microorganisms are used as organisms in the process according to the invention, they are grown or cultured in the manner with which the skilled worker is familiar, depending on the host organism. As a rule, microorganisms are grown in a liquid medium comprising a carbon source, usually in the form of sugars, a nitrogen source, usually in the form of organic nitrogen sources such as yeast extract or salts such as ammonium sulfate, trace elements such as salts of iron, manganese and magnesium and, if appropriate, vitamins, at temperatures of between 0°C and 100°C, preferably between 10°C and 60°C, while passing in oxygen. The pH of the liquid medium can either be kept constant, that is to say regulated during the culturing period, or not. The

cultures can be grown batchwise, semi-batchwise or continuously. Nutrients can be provided at the beginning of the fermentation or fed in semicontinuously or continuously. The polyunsaturated fatty acids produced can be isolated from the organisms as described above by processes known to the skilled worker, for example by extraction, distillation, crystallization, if appropriate precipitation with salt, and/or chromatography. To this end, the organisms can advantageously be disrupted beforehand.

If the host organisms are microorganisms, the process according to the invention is advantageously carried out at a temperature of between 0°C and 95°C, preferably between 10°C and 85°C, especially preferably between 15°C and 75°C, very especially preferably between 15°C and 45°C.

In this process, the pH value is advantageously kept between pH 4 and 12, preferably between pH 6 and 9, especially preferably between pH 7 and 8.

The process according to the invention can be operated batchwise, semibatchwise or continuously. An overview over known cultivation methods can be found in the textbook by Chmiel (Bioprozeßtechnik 1. Einführung in die Bioverfahrenstechnik [Bioprocess technology 1. Introduction to Bioprocess technology] (Gustav Fischer Verlag, Stuttgart, 1991)) or in the textbook by Storhas (Bioreaktoren und periphere Einrichtungen [Bioreactors and peripheral equipment] (Vieweg Verlag, Braunschweig/Wiesbaden, 1994)).

The culture medium to be used must suitably meet the requirements of the strains in question. Descriptions of culture media for various microorganisms can be found in the textbook "Manual of Methods für General Bacteriology" of the American Society for Bacteriology (Washington D. C., USA, 1981).

As described above, these media which can be employed in accordance with the invention usually comprise one or more carbon sources, nitrogen sources, inorganic salts, vitamins and/or trace elements.

Preferred carbon sources are sugars, such as mono-, di- or polysaccharides. Examples of very good carbon sources are glucose, fructose, mannose, galactose, ribose, sorbose, ribulose, lactose, maltose, sucrose, raffinose, starch or cellulose. Sugars can also be added to the media via complex compounds such as molasses or other by-products from sugar raffination. The addition of mixtures of a variety of carbon sources may also be advantageous. Other possible carbon sources are oils and fats such as, for example, soya oil, sunflower oil, peanut oil and/or coconut fat, fatty acids such as, for example, palmitic acid, stearic acid and/or linoleic acid, alcohols and/or polyalcohols such as, for example, glycerol, methanol and/or ethanol, and/or organic acids such as, for example, acetic acid and/or lactic acid.

Nitrogen sources are usually organic or inorganic nitrogen compounds or materials comprising these compounds. Examples of nitrogen sources comprise ammonia in

liquid or gaseous form or ammonium salts such as ammonium sulfate, ammonium chloride, ammonium phosphate, ammonium carbonate or ammonium nitrate, nitrates, urea, amino acids or complex nitrogen sources such as cornsteep liquor, soya meal, soya protein, yeast extract, meat extract and others. The nitrogen sources can be used individually or as a mixture.

Inorganic salt compounds which may be present in the media comprise the chloride, phosphorus and sulfate salts of calcium, magnesium, sodium, cobalt, molybdenum, potassium, manganese, zinc, copper and iron.

Inorganic sulfur-containing compounds such as, for example, sulfates, sulfites, dithionites, tetrathionates, thiosulfates, sulfides, or else organic sulfur compounds such as mercaptans and thiols may be used as sources of sulfur for the production of sulfur-containing fine chemicals, in particular of methionine.

Phosphoric acid, potassium dihydrogen phosphate or dipotassium hydrogen phosphate or the corresponding sodium-containing salts may be used as sources of phosphorus.

Chelating agents may be added to the medium in order to keep the metal ions in solution. Particularly suitable chelating agents include dihydroxyphenols such as catechol or protocatechuate and organic acids such as citric acid.

The fermentation media used according to the invention for culturing microorganisms usually also comprise other growth factors such as vitamins or growth promoters, which include, for example, biotin, riboflavin, thiamine, folic acid, nicotinic acid, panthothenate and pyridoxine. Growth factors and salts are frequently derived from complex media components such as yeast extract, molasses, cornsteep liquor and the like. It is moreover possible to add suitable precursors to the culture medium. The exact composition of the media compounds heavily depends on the particular experiment and is decided upon individually for each specific case. Information on the optimization of media can be found in the textbook "Applied Microbiol. Physiology, A Practical Approach" (Editors P.M. Rhodes, P.F. Stanbury, IRL Press (1997) pp. 53-73, ISBN 0 19 963577 3). Growth media can also be obtained from commercial suppliers, for example Standard 1 (Merck) or BHI (brain heart infusion, DIFCO) and the like.

All media components are sterilized, either by heat (20 min at 1.5 bar and 121°C) or by filter sterilization. The components may be sterilized either together or, if required, separately. All media components may be present at the start of the cultivation or added continuously or batchwise, as desired.

The culture temperature is normally between 15°C and 45°C, preferably at from 25°C to 40°C, and may be kept constant or may be altered during the experiment. The pH of the medium should be in the range from 5 to 8.5, preferably around 7.0. The pH for cultivation can be controlled during cultivation by adding basic compounds such as sodium hydroxide, potassium hydroxide, ammonia and aqueous ammonia or acidic compounds such as phosphoric acid or sulfuric acid. Foaming can be controlled by

employing antifoams such as, for example, fatty acid polyglycol esters. To maintain the stability of plasmids it is possible to add to the medium suitable substances having a selective effect, for example antibiotics. Aerobic conditions are maintained by introducing oxygen or oxygen-containing gas mixtures such as, for example, ambient air into the culture. The temperature of the culture is normally 20° to 40°C and preferably 25°C to 40°C. The culture is continued until formation of the desired product is at a maximum. This aim is normally achieved within 10 to 160 hours.

The fermentation broths obtained in this way, in particular those containing polyunsaturated fatty acids, usually contain a dry mass of from 7.5 to 25% by weight.

The fermentation broth can then be processed further. The biomass may, according to requirement, be removed completely or partially from the fermentation broth by separation methods such as, for example, centrifugation, filtration, decanting or a combination of these methods or be left completely in said broth. It is advantageous to process the biomass after its separation.

However, the fermentation broth can also be thickened or concentrated without separating the cells, using known methods such as, for example, with the aid of a rotary evaporator, thin-film evaporator, falling-film evaporator, by reverse osmosis or by nanofiltration. Finally, this concentrated fermentation broth can be processed to obtain the fatty acids present therein.

The fatty acids obtained in the process are also suitable as starting material for the chemical synthesis of further products of interest. For example, they can be used in combination with one another or alone for the preparation of pharmaceuticals, foodstuffs, animal feeds or cosmetics.

The invention furthermore relates to isolated nucleic acid sequences encoding polypeptides with Δ5-elongase, where the Δ5-elongases encoded by the nucleic acid sequences convert C_{20}-fatty acids with at least four double bonds in the fatty acid molecule, which are advantageously eventually incorporated into diacylglycerides and/or triacylglycerides.

Advantageous isolated nucleic acid sequences are nucleic acid sequences which encode polypeptides with Δ5-elongase activity and which comprise an amino acid sequence selected from the group of an amino acid sequence with the sequence shown in SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141 or SEQ ID NO: 142.

Further advantageous isolated nucleic acid sequences are nucleic acid sequences which encode polypeptides with Δ5-elongase activity and which comprise a combination of the amino acid sequences selected from the group consisting of:

a) SEQ ID NO: 115 and SEQ ID NO: 139, SEQ ID NO: 115 and SEQ ID NO: 140 or SEQ ID NO: 139 and SEQ ID NO: 140; or

b) SEQ ID NO: 116 and SEQ ID NO: 141, SEQ ID NO: 116 and SEQ ID NO: 142 or SEQ ID NO: 141 and SEQ ID NO: 142; or

c) SEQ ID NO: 115, SEQ ID NO: 139 and SEQ ID NO: 140 or SEQ ID NO: 116, SEQ ID NO: 141 and SEQ ID NO: 142.

The sequences shown in the sequences SEQ ID NO: 115 (NXXXHXXMYXYYX), SEQ ID NO: 116 (HHXXXXWAWW), SEQ ID NO: 139 (LHXXHH), SEQ ID NO: 140 (TXXQXXQF), SEQ ID NO: 141 (DTXFMV) and SEQ ID NO: 142 (TQAQXXQF) constitute conserved regions of the various elongases. Table 2 shows the meaning of the amino acids maked with X, which are present in the abovementioned nucleic acid sequences (column 3). The preferred amino acids in the various positions can also be found in the table (column 3). Column 1 indicates the SEQ ID NO, column 2 the position in the sequence.

Table 2: Meaning of the amino acid marked X in the consensus sequences.

SEQ ID NO:	Position of the X in the sequence	Amino acid	Preferred amino acid
115 (NXXXHXXMYXYYX)	2	Ser, Cys, Leu, Gly	Cys, Leu
115	3	Thr, Phe, Ile, Ser, Val, Trp, Gly	Phe, Trp
115	4	Val, Ile	Val, Ile
115	6	Val, Ile, Thr	Val, Ile
115	7	Ile, Phe, Val, Leu, Cys	Cys, Val
115	10	Ser, Gly, Tyr, Thr, Ala	Thr, Ser
115	13	Phe, Met, Thr, Leu, Ala, Gly	Leu
116 (HHXXXXWAWW)	3	Ala, Ser, Thr	Ala, Ser especially preferably Ala
116	4	Thr, Met, Val, Leu, Ile, Ser	Leu, Thr especially preferably Leu

SEQ ID NO:	Position of the X in the sequence	Amino acid	Preferred amino acid
116	5	Val, Thr, Met, Leu, Ile	Ile, Ser especially preferably Ile
116	6	Val, Met, Leu, Ile, Ala, Pro, Ser, Phe	Ile, Ser especially preferably Ile
139 LHXXHH	3	Val, Tyr, Ile	Val, Thr
139	4	Tyr, Phe	Tyr
140 TXXQXXQF	2	Asn, Asp, Thr, Gln, Met, Ser, Ala	Gln
140	3	Thr, Cys, Leu, Met, Ala, Ile, Val, Phe	Ala, Met
140	5	Met, Ile, Leu	Met
140	6	Val, Ile, Leu, Thr, Phe	Leu
141 DTXFMV	3	Leu, Ile, Val, Tyr, Phe, Ala	Phe
142 TQAQXXQF	5	Met, Ile, Leu	Met, Leu especially preferably Met
142	6	Val, Ile, Leu, Thr, Phe	Leu

Especially advantageous Δ5-elongases comprise at least one of the sequences SEQ ID NO: 116, SEQ ID NO: 141 and/or SEQ ID NO: 142.

Especially advantageous isolated nucleic acid sequences are sequences selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63; SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 113, SEQ ID NO: 131 or

SEQ ID NO: 133,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino sequence shown in SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 114, SEQ ID NO: 132 or SEQ ID NO: 134, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63; SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 113, SEQ ID NO: 131 or SEQ ID NO: 133, which encode polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 113, SEQ ID NO: 131 or SEQ ID NO: 133 and which have Δ5-elongase activity.

The invention furthermore relates to isolated nucleic acid sequences which encode polypeptides with Δ6-elongase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 69, SEQ ID NO: 81, SEQ ID NO: 111 or SEQ ID NO: 183,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 70, SEQ ID NO: 82, SEQ ID NO: 112 or SEQ ID NO: 184, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 69, SEQ ID NO: 81, SEQ ID NO: 111 or SEQ ID NO: 183 which encode polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 70, SEQ ID NO: 82, SEQ ID NO: 112 or SEQ ID NO: 184 and which have Δ6-elongase activity.

The invention furthermore relates to isolated nucleic acid sequences which encode polypeptides with ω3-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 87 or SEQ ID NO: 105,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 88 or SEQ ID NO: 106, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 87 or SEQ ID

NO: 105 which have polypeptides with at least 60% identity at the amino acid level with SEQ ID NO: 88 or SEQ ID NO: 106 and which have ω3-desaturase activity.

The invention furthermore relates to isolated nucleic acid sequences encoding a polypeptide with Δ6-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 89 or in SEQ ID NO: 97, or

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 90 or SEQ ID NO: 98, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 89 or SEQ ID NO: 97 which encode polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 90 or SEQ ID NO: 98 and which have Δ6-desaturase activity.

The invention furthermore relates to isolated nucleic acid sequences encoding a polypeptide with Δ5-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 99 or in SEQ ID NO: 101,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 100 or in SEQ ID NO: 102, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 99 or in SEQ ID NO: 101 which encode polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 100 or in SEQ ID NO: 102 and which have Δ5-desaturase activity.

The invention furthermore relates to isolated nucleic acid sequences encoding a polypeptide with Δ4-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 95 or in SEQ ID NO: 103,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 96 or SEQ ID NO: 104, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 95 or SEQ ID NO: 103 which encode polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 96 or SEQ ID NO: 104 and which have

Δ4-desaturase activity.

The invention furthermore relates to isolated nucleic acid sequences encoding a polypeptide with Δ12-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 107 or in SEQ ID NO: 109,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 108 or SEQ ID NO: 110, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 107 or SEQ ID NO: 109 which encode polypeptides with at least 50% homology at the amino acid level with SEQ ID NO: 108 or SEQ ID NO: 110 and which have Δ12-desaturase activity.

The invention furthermore relates to gene constructs which comprise the nucleic acid sequences SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183, according to the invention, wherein the nucleic acid is linked operably with one or more regulatory signals. In addition, additional biosynthesis genes of the fatty acid or lipid metabolism selected from the group acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl–ACP thioesterase(s),fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferase(s), fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl–coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenases, lipoxygenases, triacylglycerol lipases, allenoxide synthases, hydroperoxide lyases or fatty acid elongase(s) may be present in the gene construct. Advantageously, biosynthesis genes of the fatty acid or lipid metabolism selected from the group Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase, Δ6-elongase, Δ9-elongase or ω3-desaturase are additionally present.

All of the nucleic acid sequences used in the process according to the invention are advantageously derived from a eukaryotic organism such as a plant, a microorganism or an animal. The nucleic acid sequences are preferably derived from the order Salmoniformes, algae such as Mantoniella, Crypthecodinium, Euglena or Ostreococcus, fungi such as the genus Phytophthora or from diatoms such as the genera Thalassiosira or Phaeodactylum.

The nucleic acid sequences used in the process which encode proteins with ω3-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase, Δ5-elongase, Δ6-elongase or Δ9-elongase activity are advantageously introduced alone or, preferably, in combination with an expression cassette (= nucleic acid construct) which makes possible the expression of the nucleic acids in an organism, advantageously a plant or a microorganism. The nucleic acid construct can comprise more than one nucleic acid sequence with an enzymatic activity, such as, for example, of a Δ12-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ5-elongase, Δ6-elongase and/or ω3-desaturase.

To introduce the nucleic acids used in the process, the latter are advantageously amplified and ligated in the known manner. Preferably, a procedure following the protocol for Pfu DNA polymerase or a Pfu/Taq DNA polymerase mixture is followed. The primers are selected taking into consideration the sequence to be amplified. The primers should advantageously be chosen in such a way that the amplificate comprises the entire codogenic sequence from the start codon to the stop codon. After the amplification, the amplificate is expediently analyzed. For example, a gel-electrophoretic separation can be carried out, which is followed by a quantitative and a qualitative analysis. Thereafter, the amplificate can be purified following a standard protocol (for example Qiagen). An aliquot of the purified amplificate is then available for the subsequent cloning step. Suitable cloning vectors are generally known to the skilled worker. These include, in particular, vectors which are capable of replication in microbial systems, that is to say mainly vectors which ensure efficient cloning in yeasts or fungi and which make possible the stable transformation of plants. Those which must be mentioned in particular are various binary and cointegrated vector systems which are suitable for the T-DNA-mediated transformation. Such vector systems are, as a rule, characterized in that they comprise at least the vir genes required for the Agrobacterium-mediated transformation and the T-DNA-delimiting sequences (T-DNA border). These vector systems advantageously also comprise further cis-regulatory regions such as promoters and terminator sequences and/or selection markers, by means of which suitably transformed organisms can be identified. While in the case of cointegrated vector systems vir genes and T-DNA sequences are arranged on the same vector, binary systems are based on at least two vectors, one of which bears vir genes, but no T-DNA, while a second one bears T-DNA, but no vir gene. Owing to this fact, the last-mentioned vectors are relatively small, easy to manipulate and to replicate both in *E. coli* and in Agrobacterium. These binary vectors include vectors from the series pBIB-HYG, pPZP, pBecks, pGreen. In accordance with the invention, Bin19, pBI101, pBinAR, pGPTV and pCAMBIA are used by preference. An overview of the binary vectors and their use is found in Hellens et al, Trends in Plant Science (2000) 5, 446–451. In order to prepare the vectors, the vectors can first be linearized with restriction endonuclease(s) and then modified enzymatically in a suitable manner. Thereafter, the vector is purified, and an aliquot is employed for the cloning step. In the cloning step, the enzymatically cleaved and, if appropriate, purified amplificate is cloned with vector fragments which have been prepared in a similar manner, using

ligase. In this context, a particular nucleic acid construct, or vector or plasmid construct, can have one or else more than one codogenic gene segment. The codogenic gene segments in these constructs are preferably linked operably with regulatory sequences. The regulatory sequences include, in particular, plant sequences such as the above-described promoters and terminator sequences. The constructs can advantageously be stably propagated in microorganisms, in particular in E. coli and Agrobacterium tumefaciens, under selective conditions and make possible the transfer of heterologous DNA into plants or microorganisms.

The nucleic acids used in the process, the inventive nucleic acids and nucleic acid constructs, can be introduced into organisms such as microorganisms or advantageously plants, advantageously using cloning vectors, and thus be used in the transformation of plants such as those which are published and cited in: Plant Molecular Biology and Biotechnology (CRC Press, Boca Raton, Florida), Chapter 6/7, p. 71-119 (1993); F.F. White, Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, 15-38; B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-143; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225. Thus, the nucleic acids, the inventive nucleic acids and nucleic acid constructs, and/or vectors used in the process can be used for the recombinant modification of a broad spectrum of organisms, advantageously plants, so that the latter become better and/or more efficient PUFA producers.

A series of mechanisms by which a modification of the Δ12-desaturase, Δ5-elongase, Δ6-elongase, Δ5-desaturase, Δ4-desaturase, Δ6-desaturase and/or ω3-desaturase protein and of the further proteins used in the process, such as Δ12-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase or Δ4-desaturase protein, is possible exist, so that the yield, production and/or production efficiency of the advantageous polyunsaturated fatty acids in a plant, preferably in an oil crop plant or a microorganism, can be influenced directly owing to this modified protein. The number or activity of the Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase proteins or genes can be increased, so that greater amounts of the gene products and, ultimately, greater amounts of the compounds of the general formula I are produced. A *de novo* synthesis in an organism which has lacked the activity and ability to biosynthesize the compounds prior to introduction of the corresponding gene(s) is also possible. This applies analogously to the combination with further desaturases or elongases or further enzymes of the fatty acid and lipid metabolism. The use of various divergent sequences, i.e. sequences which differ at the DNA sequence level, may also be advantageous in this context, or else the use of promoters for gene expression which make possible a different gene expression in the course of time, for example as a function of the degree of maturity of a seed or an oil-storing tissue.

Owing to the introduction of a Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase and/or Δ4-desaturase gene into an organism, alone or in combination with other genes in a cell, it is not only possible to increase biosynthesis flux towards the end product, but also to increase, or to create *de novo* the corresponding triacylglycerol composition. Likewise, the number or activity of other genes which are involved in the import of nutrients which are required for the biosynthesis of one or more fatty acids, oils, polar and/or neutral lipids, can be increased, so that the concentration of these precursors, cofactors or intermediates within the cells or within the storage compartment is increased, whereby the ability of the cells to produce PUFAs as described below is enhanced further. By optimizing the activity or increasing the number of one or more Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase genes which are involved in the biosynthesis of these compounds, or by destroying the activity of one or more genes which are involved in the degradation of these compounds, an enhanced yield, production and/or efficiency of production of fatty acid and lipid molecules in organisms, advantageously in plants, is made possible.

The isolated nucleic acid molecules used in the process according to the invention encode proteins or parts of these, where the proteins or the individual protein or parts thereof comprise(s) an amino acid sequence with sufficient homology to an amino acid sequence which is shown in the sequences SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138 or SEQ ID NO: 184 so that the proteins or parts thereof retain a Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase activity. The proteins or parts thereof which is/are encoded by the nucleic acid molecule(s) preferably retains their essential enzymatic activity and the ability of participating in the metabolism of compounds required for the synthesis of cell membranes or lipid bodies in organisms, advantageously in plants, or in the transport of molecules across these membranes. Advantageously, the proteins encoded by the nucleic acid molecules have at least approximately 50%, preferably at least approximately 60% and more preferably at least approximately 70%, 80% or 90% and most preferably at least approximately 85%,

86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identity with the amino acid sequences shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138 or SEQ ID NO: 184. For the purposes of the invention, homology or homologous is understood as meaning identity or identical, respectively.

The homology was calculated over the entire amino acid or nucleic acid sequence region. The skilled worker has available a series of programs which are based on various algorithms for the comparison of various sequences. Here, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. The program PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5 1989: 151–153) or the programs Gap and BestFit [Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981)], which are part of the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)], were used for the sequence alignment. The sequence homology values which are indicated above as a percentage were determined over the entire sequence region using the program GAP and the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000. Unless otherwise specified, these settings were always used as standard settings for the sequence alignments.

Essential enzymatic activity of the Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase used in the process according to the invention is understood as meaning that they retain at least an enzymatic activity of at least 10%, preferably 20%, especially preferably 30% and very especially 40% in comparison with the proteins/enzymes encoded by the sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53,

SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 and their derivatives and can thus participate in the metabolism of compounds required for the synthesis of fatty acids, fatty acid esters such as diacylglycerides and/or triacylglycerides in an organism, advantageously a plant or a plant cell, or in the transport of molecules across membranes, meaning C_{18}-, C_{20}- or C_{22}-carbon chains in the fatty acid molecule with double bonds at at least two, advantageously three, four, five or six positions.

Nucleic acids which can advantageously be used in the process are derived from bacteria, fungi, diatoms, animals such as Caenorhabditis or Oncorhynchus or plants such as algae or mosses, such as the genera Shewanella, Physcomitrella, Thraustochytrium, Fusarium, Phytophthora, Ceratodon, Mantoniella, Ostreococcus, Isochrysis, Aleurita, Muscarioides, Mortierella, Borago, Phaeodactylum, Crypthecodinium, specifically from the genera and species Oncorhynchus mykiss, Xenopus laevis, Ciona intestinalis, Thalassiosira pseudonona, Mantoniella squamata, Ostreococcus sp., Ostreococcus tauri, Euglena gracilis, Physcomitrella patens, Phytophtora infestans, Fusarium graminaeum, Cryptocodinium cohnii, Ceratodon purpureus, Isochrysis galbana, Aleurita farinosa, Thraustochytrium sp., Muscarioides viallii, Mortierella alpina, Borago officinalis, Phaeodactylum tricornutum, Caenorhabditis elegans or especially advantageously from Oncorhynchus mykiss, Euglena gracilis, Thalassiosira pseudonona or Crypthecodinium cohnii.

Alternatively, nucleic acid sequences which encode a Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase and which advantageously hybridize under stringent conditions with a nucleic acid sequence as shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 can be used in the process

according to the invention.

The nucleic acid sequences used in the process are advantageously introduced into an expression cassette which makes possible the expression of the nucleic acids in organisms such as microorganisms or plants.

In doing so, the nucleic acid sequences which encode Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase are linked operably with one or more regulatory signals, advantageously for enhancing gene expression. These regulatory sequences are intended to make possible the specific expression of the genes and proteins. Depending on the host organism, this may mean, for example, that the gene is expressed and/or overexpressed only after induction has taken place, or else that it is expressed and/or overexpressed immediately. For example, these regulatory sequences take the form of sequences to which inductors or repressors bind, thus controlling the expression of the nucleic acid. In addition to these novel regulatory sequences, or instead of these sequences, the natural regulatory elements of these sequences may still be present before the actual structural genes and, if appropriate, may have been genetically modified in such a way that their natural regulation is eliminated and the expression of the genes is enhanced. However, the expression cassette (= expression construct = gene construct) can also be simpler in construction, that is to say no additional regulatory signals have been inserted before the nucleic acid sequence or its derivatives, and the natural promoter together with its regulation was not removed. Instead, the natural regulatory sequence has been mutated in such a way that regulation no longer takes place and/or gene expression is enhanced. These modified promoters can also be positioned on their own before the natural gene in the form of part-sequences (= promotor with parts of the nucleic acid sequences used in accordance with the invention) in order to enhance the activity. Moreover, the gene construct may advantageously also comprise one or more what are known as enhancer sequences in operable linkage with the promoter, which make possible an enhanced expression of the nucleic acid sequence. Additional advantageous sequences, such as further regulatory elements or terminator sequences, may also be inserted at the 3' end of the DNA sequences. The Δ12-desaturase, ω3-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ5-elongase, Δ6-elongase and/or Δ9-elongase genes may be present in one or more copies of the expression cassette (= gene construct). Preferably, only one copy of the genes is present in each expression cassette. This gene construct or the gene constructs can be expressed together in the host organism. In this context, the gene construct(s) can be inserted in one or more vectors and be present in the cell in free form, or else be inserted in the genome. It is advantageous for the insertion of further genes in the genome when the genes to be expressed are present together in one gene construct.

In this context, the regulatory sequences or factors can, as described above, preferably have a positive effect on the gene expression of the genes introduced, thus enhancing it. Thus, an enhancement of the regulatory elements, advantageously at the

transcriptional level, may take place by using strong transcription signals such as promoters and/or enhancers. In addition, however, enhanced translation is also possible, for example by improving the stability of the mRNA.

A further embodiment of the invention is one or more gene constructs which comprise one or more sequences which are defined by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 or its derivatives and which encode polypeptides as shown in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138 or SEQ ID NO: 184. The abovementioned Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase proteins lead advantageously to a desaturation or elongation of fatty acids, the substrate advantageously having one, two, three, four, five or six double bonds and advantageously 18, 20 or 22 carbon atoms in the fatty acid molecule. The same applies to their homologs, derivatives or analogs, which are linked operably with one or more regulatory signals, advantageously for enhancing gene expression.

Advantageous regulatory sequences for the novel process are present for example in promoters such as the cos, tac, trp, tet, trp-tet, lpp, lac, lpp-lac, laclq, T7, T5, T3, gal, trc, ara, SP6, λ-PR or λ-PL promoter and are advantageously employed in Gram-negative bacteria. Further advantageous regulator sequences are, for example,

present in the Gram-positive promoters amy and SPO2, in the yeast or fungal promoters ADC1, MFα, AC, P-60, CYC1, GAPDH, TEF, rp28, ADH or in the plant promoters CaMV/35S [Franck et al., Cell 21 (1980) 285–294], PRP1 [Ward et al., Plant. Mol. Biol. 22 (1993)], SSU, OCS, lib4, usp, STLS1, B33, nos or in the ubiquitin or phaseolin promoter. Advantageous in this context are also inducible promoters, such as the promoters described in EP-A-0 388 186 (benzenesulfonamide-inducible), Plant J. 2, 1992:397–404 (Gatz et al., tetracycline-inducible), EP–A–0 335 528 (abscissic acid-inducible) or WO 93/21334 (ethanol- or cyclohexenol-inducible) promoters. Further suitable plant promoters are the cytosolic FBPase promoter or the ST-LSI promoter of potato (Stockhaus et al., EMBO J. 8, 1989, 2445), the glycine max phosphoribosylpyrophosphate amidotransferase promoter (Genbank Accession No.. U87999) or the node-specific promoter described in EP–A–0 249 676.

Especially advantageous promoters are promoters which make possible the expression in tissues which are involved in the biosynthesis of fatty acids. Very especially advantageous are seed-specific promoters, such as the USP promoter as described, but also other promoters such as the LeB4, DC3, phaseolin or napin promoter. Further especially advantageous promoters are seed-specific promoters which can be used for monocotyledonous or dicotyledonous plants and which are described in US 5,608,152 (oilseed rape napin promoter), WO 98/45461 (Arabidopsis oleosin promoter), US 5,504,200 (Phaseolus vulgaris phaseolin promoter), WO 91/13980 (Brassica Bce4 promoter), by Baeumlein et al., Plant J., 2, 2, 1992:233–239 (LeB4 promoter from a legume), these promoters being suitable for dicots. Examples of promoters which are suitable for monocots are the barley lpt–2 or lpt–1 promoter (WO 95/15389 and WO 95/23230), the barley hordein promoter and other suitable promoters described in WO 99/16890.

In principle, it is possible to use all natural promoters together with their regulatory sequences, such as those mentioned above, for the novel process. It is also possible and advantageous to use synthetic promoters, either in addition or alone, in particular when they mediate seed-specific expression, such as those described in WO 99/16890.

In order to achieve a particularly high PUFA content, especially in transgenic plants, the PUFA biosynthesis genes should advantageously be expressed in oil crops in a seed-specific manner. To this end, seed-specific promoters can be used, or those promoters which are active in the embryo and/or in the endosperm. In principle, seed-specific promoters can be isolated both from dicotyledonous and from monocotyledonous plants. Preferred promoters are listed hereinbelow: USP (= unknown seed protein) and vicilin (Vicia faba) [Bäumlein et al., Mol. Gen Genet., 1991, 225(3)], napin (oilseed rape) [US 5,608,152], acyl carrier protein (oilseed rape) [US 5,315,001 and WO 92/18634], oleosin (Arabidopsis thaliana) [WO 98/45461 and WO 93/20216], phaseolin (Phaseolus vulgaris) [US 5,504,200], Bce4 [WO 91/13980], legumines B4 (LegB4 promoter) [Bäumlein et al., Plant J., 2,2, 1992], Lpt2 and lpt1 (barley) [WO 95/15389 and WO95/23230], seed-specific promoters from rice, maize

and wheat [WO 99/16890], Amy32b, Amy 6-6 and aleurain [US 5,677,474], Bce4 (oilseed rape) [US 5,530,149], glycinin (soybean) [EP 571 741], phosphoenol pyruvate carboxylase (soybean) [JP 06/62870], ADR12-2 (soybean) [WO 98/08962], isocitrate lyase (oilseed rape) [US 5,689,040] or α-amylase (barley) [EP 781 849].

Plant gene expression can also be facilitated via a chemically inducible promoter (see review in Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly suitable when it is desired that gene expression should take place in a time-specific manner. Examples of such promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracycline-inducible promoter (Gatz et al. (1992) Plant J. 2, 397-404) and an ethanol-inducible promoter.

To ensure the stable integration of the biosynthesis genes into the transgenic plant over a plurality of generations, each of the nucleic acids which encode Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase and/or Δ4-desaturase and which are used in the process should be expressed under the control of a separate promoter, preferably a promoter which differs from the other promoters, since repeating sequence motifs can lead to instability of the T-DNA, or to recombination events. In this context, the expression cassette is advantageously constructed in such a way that a promoter is followed by a suitable cleavage site, advantageously in a polylinker, for insertion of the nucleic acid to be expressed and, if appropriate, a terminator sequence is positioned behind the polylinker. This sequence is repeated several times, preferably three, four or five times, so that up to five genes can be combined in one construct and introduced into the transgenic plant in order to be expressed. Advantageously, the sequence is repeated up to three times. To express the nucleic acid sequences, the latter are inserted behind the promoter via a suitable cleavage site, for example in the polylinker. Advantageously, each nucleic acid sequence has its own promoter and, if appropriate, its own terminator sequence. Such advantageous constructs are disclosed, for example, in DE 101 02 337 or DE 101 02 338. However, it is also possible to insert a plurality of nucleic acid sequences behind a promoter and, if appropriate, before a terminator sequence. Here, the insertion site, or the sequence, of the inserted nucleic acids in the expression cassette is not of critical importance, that is to say a nucleic acid sequence can be inserted at the first or last position in the cassette without its expression being substantially influenced thereby. Advantageously, different promoters such as, for example, the USP, LegB4 or DC3 promoter, and different terminator sequences can be used in the expression cassette. However, it is also possible to use only one type of promoter in the cassette. This, however, may lead to undesired recombination events.

As described above, the transcription of the genes which have been introduced should advantageously be terminated by suitable terminator sequences at the 3' end of the biosynthesis genes which have been introduced (behind the stop codon). An example of a sequence which can be used in this context is the OCS 1 terminator sequence. As is the case with the promoters, different terminator sequences should be used for each

gene.

As described above, the gene construct can also comprise further genes to be introduced into the organisms. It is possible and advantageous to introduce into the host organisms, and to express therein, regulatory genes such as genes for inductors, repressors or enzymes which, owing to their enzyme activity, engage in the regulation of one or more genes of a biosynthesis pathway. These genes can be of heterologous or of homologous origin. Moreover, further biosynthesis genes of the fatty acid or lipid metabolism can advantageously be present in a nucleic acid construct, or gene construct; however, these genes can also be positioned on one or more further nucleic acid constructs. Biosynthesis genes of the fatty acid or lipid metabolism which are preferably used is a gene selected from the group consisting of acyl-CoA dehydrogenase(s), acyl-ACP [= acyl carrier protein] desaturase(s), acyl-ACP thioesterase(s), fatty acid acyltransferase(s), acyl-CoA:lysophospholipid acyltransferases, fatty acid synthase(s), fatty acid hydroxylase(s), acetyl-coenzyme A carboxylase(s), acyl-coenzyme A oxidase(s), fatty acid desaturase(s), fatty acid acetylenase(s), lipoxygenase(s), triacylglycerol lipase(s), allenoxide synthase(s), hydroperoxide lyase(s) or fatty acid elongase(s) or combinations thereof. Especially advantageous nucleic acid sequences are biosynthesis genes of the fatty acid or lipid metabolism selected from the group of the acyl-CoA:lysophospholipid acyltransferase, ω3-desaturase, Δ4-desaturase, Δ5-desaturase, Δ6-desaturase, Δ8-desaturase, Δ9-desaturase, Δ12-desaturase, Δ5-elongase, Δ6-elongase and/or Δ9-elongase.

In this context, the abovementioned nucleic acids or genes can be cloned into expression cassettes, like those mentioned above, in combination with other elongases and desaturases and used for transforming plants with the aid of Agrobacterium.

Here, the regulatory sequences or factors can, as described above, preferably have a positive effect on, and thus enhance, the expression genes which have been introduced. Thus, enhancement of the regulatory elements can advantageously take place at the transcriptional level by using strong transcription signals such as promoters and/or enhancers. However, an enhanced translation is also possible, for example by improving the stability of the mRNA. In principle, the expression cassettes can be used directly for introduction into the plants or else be introduced into a vector.

These advantageous vectors, preferably expression vectors, comprise the nucleic acids which encode the Δ12-desaturases, ω3-desaturases, Δ9-elongases, Δ6-desaturases, Δ8-desaturases, Δ6-elongases, Δ5-desaturases, Δ5-elongases or Δ4-desaturases and which are used in the process, or else a nucleic acid construct which the nucleic acid used either alone or in combination with further biosynthesis genes of the fatty acid or lipid metabolism such as the acyl-CoA:lysophospholipid acyltransferases, ω3-desaturases, Δ4-desaturases, Δ5-desaturases, Δ6-desaturases, Δ8-desaturases, Δ9-desaturases, Δ12-desaturases, ω3-desaturases, Δ5-elongases, Δ6-elongases and/or Δ9-elongases. As used in the present context, the term "vector" refers to a nucleic acid molecule which is capable of transporting another nucleic acid

to which it is bound. One type of vector is a "plasmid", a circular double-stranded DNA loop into which additional DNA segments can be ligated. A further type of vector is a viral vector, it being possible for additional DNA segments to be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they have been introduced (for example bacterial vectors with bacterial replication origin). Other vectors are advantageously integrated into the genome of a host cell when they are introduced into the host cell, and thus replicate together with the host genome. Moreover, certain vectors can govern the expression of genes with which they are in operable linkage. These vectors are referred to in the present context as "expression vectors". Usually, expression vectors which are suitable for DNA recombination techniques take the form of plasmids. In the present description, "plasmid" and "vector" can be used exchangeably since the plasmid is the form of vector which is most frequently used. However, the invention is also intended to cover other forms of expression vectors, such as viral vectors, which exert similar functions. Furthermore, the term "vector" is also intended to comprise other vectors with which the skilled worker is familiar, such as phages, viruses such as SV40, CMV, TMV, transposons, IS elements, phasmids, phagemids, cosmids, linear or circular DNA.

The recombinant expression vectors advantageously used in the process comprise the nucleic acids described below or the above-described gene construct in a form which is suitable for expressing the nucleic acids used in a host cell, which means that the recombinant expression vectors comprise one or more regulatory sequences, selected on the basis of the host cells used for the expression, which regulatory sequence(s) is/are linked operably with the nucleic acid sequence to be expressed. In a recombinant expression vector, "linked operably" means that the nucleotide sequence of interest is bound to the regulatory sequence(s) in such a way that the expression of the nucleotide sequence is possible and they are bound to each other in such a way that both sequences carry out the predicted function which is ascribed to the sequence (for example in an in-vitro transcription/translation system, or in a host cell if the vector is introduced into the host cell). The term "regulatory sequence" is intended to comprise promoters, enhancers and other expression control elements (for example polyadenylation signals). These regulatory sequences are described, for example, in Goeddel: Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990), or see: Gruber and Crosby, in: Methods in Plant Molecular Biology and Biotechnolgy, CRC Press, Boca Raton, Florida, Ed.: Glick and Thompson, Chapter 7, 89-108, including the references cited therein. Regulatory sequences comprise those which govern the constitutive expression of a nucleotide sequence in many types of host cell and those which govern the direct expression of the nucleotide sequence only in specific host cells under specific conditions. The skilled worker knows that the design of the expression vector can depend on factors such as the choice of host cell to be transformed, the desired expression level of the protein and the like.

The recombinant expression vectors used can be designed for the expression of Δ12-desaturases, ω3-desaturases, Δ9-elongases, Δ6-desaturases, Δ8-desaturases, Δ6-elongases, Δ5-desaturases, Δ5-elongases and/or Δ4-desaturases in prokaryotic or

eukaryotic cells. This is advantageous since intermediate steps of the vector construction are frequently carried out in microorganisms for the sake of simplicity. For example, the Δ12-desaturase, ω3-desaturases, Δ9-elongases, Δ6-desaturase, Δ8-desaturases, Δ6-elongase, Δ5-desaturase, Δ5-elongase and/or Δ4-desaturase genes can be expressed in bacterial cells, insect cells (using Baculovirus expression vectors), yeast and other fungal cells (see Romanos, M.A., et al. (1992) "Foreign gene expression in yeast: a review", Yeast 8:423-488; van den Hondel, C.A.M.J.J., et al. (1991) "Heterologous gene expression in filamentous fungi", in: More Gene Manipulations in Fungi, J.W. Bennet & L.L. Lasure, Ed., pp. 396-428: Academic Press: San Diego; and van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of Fungi, Peberdy, J.F., et al., Ed., pp. 1-28, Cambridge University Press: Cambridge), algae (Falciatore et al., 1999, Marine Biotechnology.1, 3:239-251), ciliates of the types: Holotrichia, Peritrichia, Spirotrichia, Suctoria, Tetrahymena, Paramecium, Colpidium, Glaucoma, Platyophrya, Potomacus, Desaturaseudocohnilembus, Euplotes, Engelmaniella and Stylonychia, in particular of the genus Stylonychia lemnae, using vectors in a transformation method as described in WO 98/01572 and, preferably, in cells of multi-celled plants (see Schmidt, R. and Willmitzer, L. (1988) "High efficiency Agrobacterium tumefaciens-mediated transformation of Arabidopsis thaliana leaf and cotyledon explants" Plant Cell Rep.:583-586; Plant Molecular Biology and Biotechnology, C Press, Boca Raton, Florida, Chapter 6/7, pp.71-119 (1993); F.F. White, B. Jenes et al., Techniques for Gene Transfer, in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press (1993), 128-43; Potrykus, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991), 205-225 (and references cited therein)). Suitable host cells are furthermore discussed in Goeddel, Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, CA (1990). As an alternative, the recombinant expression vector can be transcribed and translated in vitro, for example using T7-promoter regulatory sequences and T7-polymerase.

In most cases, the expression of proteins in prokaryotes involves the use of vectors comprising constitutive or inducible promoters which govern the expression of fusion or nonfusion proteins. Typical fusion expression vectors are, inter alia, pGEX (Pharmacia Biotech Inc; Smith, D.B., and Johnson, K.S. (1988) Gene 67:31-40), pMAL (New England Biolabs, Beverly, MA) und pRIT5 (Pharmacia, Piscataway, NJ), where glutathione S-transferase (GST), maltose-E binding protein and protein A, respectively, is fused with the recombinant target protein.

Examples of suitable inducible nonfusion E. coli expression vectors are, inter alia, pTrc (Amann et al. (1988) Gene 69:301-315) and pET 11d (Studier et al., Gene Expression Technology: Methods in Enzymology 185, Academic Press, San Diego, California (1990) 60-89). The target gene expression from the pTrc vector is based on the transcription from a hybrid trp-lac fusion promoter by the host RNA polymerase. The target gene expression from the vector pET 11d is based on the transcription of a

T7-gn10-lac fusion promoter, which is mediated by a viral RNA polymerase (T7 gn1), which is coexpressed. This viral polymerase is provided by the host strains BL21 (DE3) or HMS174 (DE3) from a resident λ-prophage which harbors a T7 gn1 gene under the transcriptional control of the lacUV 5 promoter.

Other vectors which are suitable for prokaryotic organisms are known to the skilled worker, these vectors are, for example in E. coli pLG338, pACYC184, the pBR series such as pBR322, the pUC series such as pUC18 or pUC19, the M113mp series, pKC30, pRep4, pHS1, pHS2, pPLc236, pMBL24, pLG200, pUR290, pIN-III113-B1, λgt11 or pBdCl, in Streptomyces pIJ101, pIJ364, pIJ702 or pIJ361, in Bacillus pUB110, pC194 or pBD214, in Corynebacterium pSA77 or pAJ667.

In a further embodiment, the expression vector is a yeast expression vector. Examples for vectors for expression in the yeast S. cerevisiae comprise pYeDesaturasec1 (Baldari et al. (1987) Embo J. 6:229-234), pMFa (Kurjan and Herskowitz (1982) Cell 30:933-943), pJRY88 (Schultz et al. (1987) Gene 54:113-123) and pYES2 (Invitrogen Corporation, San Diego, CA). Vectors and processes for the construction of vectors which are suitable for use in other fungi, such as the filamentous fungi, comprise those which are described in detail in: van den Hondel, C.A.M.J.J., & Punt, P.J. (1991) "Gene transfer systems and vector development for filamentous fungi, in: Applied Molecular Genetics of fungi, J.F. Peberdy et al., Ed., pp. 1-28, Cambridge University Press: Cambridge, or in: More Gene Manipulations in Fungi [J.W. Bennet & L.L. Lasure, Ed., pp. 396-428: Academic Press: San Diego]. Further suitable yeast vectors are, for example, pAG-1, YEp6, YEp13 or pEMBLYe23.

As an alternative, Δ12-desaturases, ω3-desaturases, Δ9-elongases, Δ6-desaturases, Δ8-desaturases, Δ6-elongases, Δ5-desaturases, Δ5-elongases and/or Δ4-desaturases can be expressed in insect cells using Baculovirus vectors. Baculovirus expression vectors which are available for the expression of proteins in cultured insect cells (for example Sf9 cells) comprise the pAc series (Smith et al. (1983) Mol. Cell Biol.. 3:2156-2165) and the pVL series (Lucklow and Summers (1989) Virology 170:31-39).

The abovementioned vectors are only a small overview over suitable vectors which are possible. Further plasmids are known to the skilled worker and are described, for example, in: Cloning Vectors (Ed. Pouwels, P.H., et al., Elsevier, Amsterdam-New York-Oxford, 1985, ISBN 0 444 904018). For further suitable expression systems for prokaryotic and eukaryotic cells, see the Chapters 16 and 17 in Sambrook, J., Fritsch, E.F., and Maniatis, T., Molecular Cloning: A Laboratory Manual, 2. edition, Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989.

In a further embodiment of the process, the Δ12-desaturases, ω3-desaturases, Δ9-elongases, Δ6-desaturases, Δ8-desaturases, Δ6-elongases, Δ5-desaturases, Δ5-elongases and/or Δ4-desaturases can be expressed in single-celled plant cells (such as algae), see Falciatore et al., 1999, Marine Biotechnology 1 (3):239-251 and

references cited therein, and in plant cells from higher plants (for example spermatophytes such as arable crops). Examples of plant expression vectors comprise those which are described in detail in: Becker, D., Kemper, E., Schell, J., and Masterson, R. (1992) "New plant binary vectors with selectable markers located proximal to the left border", Plant Mol. Biol. 20:1195-1197; and Bevan, M.W. (1984) "Binary Agrobacterium vectors for plant transformation", Nucl. Acids Res. 12:8711-8721; Vectors for Gene Transfer in Higher Plants; in: Transgenic Plants, Vol. 1, Engineering and Utilization, Ed.: Kung and R. Wu, Academic Press, 1993, p. 15-38.

A plant expression cassette preferably comprises regulatory sequences which are capable of governing the expression of genes in plant cells and which are linked operably so that each sequence can fulfill its function, such as transcriptional termination, for example polyadenylation signals. Preferred polyadenylation signals are those which are derived from Agrobacterium tumefaciens T-DNA, such as gene 3 of the Ti plasmid pTiACH5 (Gielen et al., EMBO J. 3 (1984) 835 et seq.), which is known as octopine synthase, or functional equivalents thereof, but all other terminator sequences which are functionally active in plants are also suitable.

Since plant gene expression is very often not limited to the transcriptional level, a plant expression cassette preferably comprises other sequences which are linked operably, such as translation enhancers, for example the overdrive sequence, which enhances the tobacco mosaic virus 5' – untranslated leader sequence, which increases the protein/RNA ratio (Gallie et al., 1987, Nucl. Acids Research 15:8693-8711).

As described above, plant gene expression must be linked operably with a suitable promoter which triggers gene expression with the correct timing or in a cell- or tissue-specific manner. Utilizable promoters are constitutive promoters (Benfey et al., EMBO J. 8 (1989) 2195-2202), such as those which are derived from plant viruses, such as 35S CaMV (Franck et al., Cell 21 (1980) 285-294), 19S CaMV (see also US 5352605 and WO 84/02913), or plant promoters, such as the promoter of the Rubisco subunit, which is described in US 4,962,028.

Other preferred sequences for use in operable linkage in plant gene expression cassettes are targeting sequences, which are required for steering the gene product into its corresponding cell compartment (see a review in Kermode, Crit. Rev. Plant Sci. 15, 4 (1996) 285-423 and references cited therein), for example into the vacuole, into the nucleus, all types of plastids, such as amyloplasts, chloroplasts, chromoplasts, the extracellular space, the mitochondria, the endoplasmid reticulum, elaioplasts, peroxisomes and other compartments of plant cells.

As described above, plant gene expression can also be achieved via a chemically inducible promoter (see review in Gatz 1997, Annu. Rev. Plant Physiol. Plant Mol. Biol., 48:89-108). Chemically inducible promoters are particularly suitable when it is desired that the gene expression takes place in a time-specific manner. Examples of such

promoters are a salicylic-acid-inducible promoter (WO 95/19443), a tetracyclin-inducible promoter (Gatz et al. (1992) Plant J. 2, 397-404) and an ethanol-inducible promoter.

Promoters which respond to biotic or abiotic stress conditions are also suitable, for example the pathogen-induced PRP1 gene promoter (Ward et al., Plant. Mol. Biol. 22 (1993) 361-366), the heat-inducible tomato hsp80 promoter (US 5,187,267), the chill-inducible potato alpha-amylase promoter (WO 96/12814) or the wound-inducible pinII promoter (EP-A-0 375 091).

Especially preferred are those promoters which bring about the gene expression in tissues and organs in which the biosynthesis of fatty acids, lipids and oils takes place, in seed cells, such as cells of the endosperm and of the developing embryo. Suitable promoters are the oilseed rape napin promoter (US 5,608,152), the Vicia faba USP promoter (Baeumlein et al., Mol Gen Genet, 1991, 225 (3):459-67), the Arabidopsis oleosin promoter (WO 98/45461), the Phaseolus vulgaris phaseolin promoter (US 5,504,200), the Brassica Bce4 promoter (WO 91/13980) or the legumine B4 promoter (LeB4; Baeumlein et al., 1992, Plant Journal, 2 (2):233-9), and promoters which bring about the seed-specific expression in monocotyledonous plants such as maize, barley, wheat, rye, rice and the like. Suitable noteworthy promoters are the barley lpt2 or lpt1 gene promoter (WO 95/15389 and WO 95/23230) or the promoters from the barley hordein gene, the rice glutelin gene, the rice oryzin gene, the rice prolamine gene, the wheat gliadine gene, the wheat glutelin gene, the maize zeine gene, the oat glutelin gene, the sorghum kasirin gene or the rye secalin gene, which are described in WO 99/16890.

In particular, it may be desired to bring about the multiparallel expression of the Δ12-desaturases, ω3-desaturases, Δ9-elongases, Δ6-desaturases, Δ8-desaturases, Δ6-elongases, Δ5-desaturases, Δ5-elongases and/or Δ4-desaturases used in the process. Such expression cassettes can be introduced via the simultaneous transformation of a plurality of individual expression constructs or, preferably, by combining a plurality of expression cassettes on one construct. Also, a plurality of vectors can be transformed with in each case a plurality of expression cassettes and then transferred into the host cell.

Other promoters which are likewise especially suitable are those which bring about a plastid-specific expression, since plastids constitute the compartment in which the precursors and some end products of lipid biosynthesis are synthesized. Suitable promoters, such as the viral RNA polymerase promoter, are described in WO 95/16783 and WO 97/06250, and the clpP promoter from Arabidopsis, described in WO 99/46394.

Vector DNA can be introduced into prokaryotic and eukaryotic cells via conventional transformation or transfection techniques. The terms “transformation” and “transfection”, conjugation and transduction, as used in the present context, are

intended to comprise a multiplicity of methods known in the prior art for the introduction of foreign nucleic acid (for example DNA) into a host cell, including calcium phosphate or calcium chloride coprecipitation, DEAE-dextran-mediated transfection, lipofection, natural competence, chemically mediated transfer, electroporation or particle bombardment. Suitable methods for the transformation or transfection of host cells, including plant cells, can be found in Sambrook et al. (Molecular Cloning: A Laboratory Manual., 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) and other laboratory textbooks such as Methods in Molecular Biology, 1995, Vol. 44, Agrobacterium protocols, Ed.: Gartland and Davey, Humana Press, Totowa, New Jersey.

Host cells which are suitable in principle for taking up the nucleic acid according to the invention, the gene product according to the invention or the vector according to the invention are all prokaryotic or eukaryotic organisms. The host organisms which are advantageously used are microorganisms such as fungi or yeasts, or plant cells, preferably plants or parts thereof. Fungi, yeasts or plants are preferably used, especially preferably plants, very especially preferably plants such as oil crops, which are high in lipid compounds, such as oilseed rape, evening primrose, hemp, thistle, peanut, canola, linseed, soybean, safflower, sunflower, borage, or plants such as maize, wheat, rye, oats, triticale, rice, barley, cotton, cassava, pepper, Tagetes, Solanacea plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, alfalfa, bushy plants (coffee, cacao, tea), Salix species, trees (oil palm, coconut), and perennial grasses and fodder crops. Especially preferred plants according to the invention are oil crops such as soybean, peanut, oilseed rape, canola, linseed, hemp, evening primrose, sunflower, safflower, trees (oil palm, coconut).

The invention furthermore relates to above-described isolated nucleic acid sequence which encode polypeptides with Δ5-elongase activity, where the elongase encoded by the nucleic acid sequences converts C_{16}- and C_{18}-fatty acids with one double bond and advantageously polyunsaturated C_{18}-fatty acids with one Δ6 double bond and polyunsaturated C_{20}-fatty acids with one Δ5 double bond. C_{22}-fatty acids are not elongated.

Advantageous isolated nucleic acid sequences are nucleic acid sequences which encode polypeptides with Δ5-elongase activity and which comprise an amino acid sequence selected from the group of an amino acid sequence with the squence shown in SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 139, SEQ ID NO: 140, SEQ ID NO: 141 or SEQ ID NO: 142.

Further advantageous isolated nucleic acid sequences are nucleic acid sequences which encode polypeptides with Δ5-elongase activity and which comprise a combination of the amino acid sequences selected from the group consisting of:

a) SEQ ID NO: 115 and SEQ ID NO: 139, SEQ ID NO: 115 and SEQ ID NO: 140 or SEQ ID NO: 139 and SEQ ID NO: 140; or

b) SEQ ID NO: 116 and SEQ ID NO: 141, SEQ ID NO: 116 and SEQ ID NO: 142 or SEQ ID NO: 141 and SEQ ID NO: 142; or

c) SEQ ID NO: 115, SEQ ID NO: 139 and SEQ ID NO: 140 or SEQ ID NO: 116, SEQ ID NO: 141 and SEQ ID NO: 142.

Preferred nucleic acid sequences which encode polypeptides with Δ5-elongase activity advantageously comprise the abovementioned amino acid sequences. The latter are described in greater detail in table 2.

Especially advantageous isolated nucleic acid sequences are sequences selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 113, SEQ ID NO: 131 or SEQ ID NO: 133,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 114, SEQ ID NO: 132 or SEQ ID NO: 134, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 113, SEQ ID NO: 131 or SEQ ID NO: 133 which have polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 114, SEQ ID NO: 132 or SEQ ID NO: 134 and which have Δ5-elongase activity.

The invention furthermore relates to the nucleic acid sequences which are enumerated hereinbelow and which encode Δ6-elongases, ω3-desaturases, Δ6-desaturases, Δ5-desaturases, Δ4-desaturases or Δ12-desaturases.

Further advantageous isolated nucleic acid sequences are sequences selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 69, SEQ ID NO: 81, SEQ ID NO: 111 or SEQ ID NO: 183,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 70, SEQ ID NO: 82, SEQ ID NO: 112 or SEQ ID NO: 184, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 69, SEQ ID NO: 81, SEQ ID NO: 111 or SEQ ID NO: 183 which encode polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 70, SEQ ID NO: 82, SEQ ID NO: 112 or SEQ ID NO: 184 and which have Δ6-elongase activity.

Isolated nucleic acid sequences encoding olypeptides with ω3-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 87 or SEQ ID NO: 105,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 88 or SEQ ID NO: 106, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 87 or SEQ ID NO: 105 which have polypeptides with at least 60% identity at the amino acid level with SEQ ID NO: 88 or SEQ ID NO: 106 and which have ω3-desaturase activity.

Isolated nucleic acid sequences encoding polypeptides with Δ6-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 89 or in SEQ ID NO: 97,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 90 or SEQ ID NO: 98, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 89 or SEQ ID NO: 97 which encode polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 90 or SEQ ID NO: 98 and which have Δ6-desaturase activity.

Isolated nucleic acid sequences encoding polypeptides with Δ5-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 99 or in SEQ ID NO: 101,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 100 or in SEQ ID NO: 102, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 99 or in SEQ ID NO: 101 which encode polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 92, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 100 or in SEQ ID NO: 102 and which have Δ5-desaturase activity.

Isolated nucleic acid sequences encoding polypeptides with Δ12-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 95 or in SEQ ID NO: 103,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 96 or in SEQ ID NO: 104, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 95 or in SEQ ID NO: 103 which encode polypeptides with at least 40% homology at the amino acid level with SEQ ID NO: 96 or in SEQ ID NO: 104 and which have Δ6-desaturase activity.

Isolated nucleic acid sequences encoding polypeptides with Δ12-desaturase activity, selected from the group consisting of:

a) a nucleic acid sequence with the sequence shown in SEQ ID NO: 107 or in SEQ ID NO: 109,

b) nucleic acid sequences which, as the result of the degeneracy of the genetic code, can be derived from the amino acid sequence shown in SEQ ID NO: 108 or SEQ ID NO: 110, or

c) derivatives of the nucleic acid sequence shown in SEQ ID NO: 107 or SEQ ID NO: 109 which encode polypeptides with at least 50% homology at the amino acid level with SEQ ID NO: 108 or SEQ ID NO: 110 and which have Δ12-desaturase activity.

The abovementioned nucleic acids according to the invention are derived from organisms such as nonhuman animals, ciliates, fungi, plants such as algae or dinoflagellates which are capable of synthesizing PUFAs.

The isolated abovementioned nucleic acid sequences are advantageously derived from the order Salmoniformes, Xenopus or Ciona, the diatom genera Thalassiosira or Crythecodinium, or from the family of the Prasinophyceae, such as the genus Ostreococcus or the family Euglenaceae, such as the genus Euglena, or Pythiaceae, such as the genus Phytophthora.

The invention furthermore relates to isolated nucleic acid sequences as described above which encode polypeptides with ω3-desaturase activity, where the ω3-desaturases encoded by the nucleic acid sequences convert C_{18}-, C_{20}- and C_{22}-fatty acids with two, three, four or five double bonds and advantageously polyunsaturated C_{18}-fatty acids with two or three double bonds and polyunsaturated C_{20}-fatty acids with two, three or four double bonds. C_{22}-Fatty acids with four or five double bonds are also desaturated.

As described above, the invention furthermore relates to isolated nucleic acid sequence which encode polypeptides with Δ12-desaturases, Δ4-desaturases, Δ5-desaturases and Δ6-desaturases, where the Δ12-desaturases, Δ4-desaturases, Δ5-desaturases or Δ6-desaturases encoded by these nucleic acid sequences convert C_{18}-, C_{20}- and C_{22}-fatty acids with one, two, three, four or five double bonds and advantageously polyunsaturated C_{18}-fatty acids with one, two or three double bonds such as $C18:1^{\Delta 9}$, $C18:2^{\Delta 9,12}$or $C18:3^{\Delta 9,12,15}$, polyunsaturated C_{20}-fatty acids with three or four double bonds such as $C20:3^{\Delta 8,11,14}$ or $C20:4^{\Delta 8,11,14,17}$ or polyunsaturated C_{22}-fatty acids with four or five double bonds such as $C22:4^{\Delta 7,10,13,16}$ or $C22:5^{\Delta 7,10,13,16,19}$. The fatty acids are advantageously desaturated in the phospholipids or CoA-fatty acid esters, advantageously in the CoA-fatty acid esters.

In an advantageous embodiment, the term "nucleic acid (molecule)" as used in the present context additionally comprises the untranslated sequence at the 3' and at the 5' end of the coding gene region: at least 500, preferably 200, especially preferably 100 nucleotides of the sequence upstream of the 5' end of the coding region and at least 100, preferably 50, especially preferably 20 nucleotides of the sequence downstream of the 3' end of the coding gene region. An "isolated" nucleic acid molecule is separate from other nucleic acid molecules which are present in the natural source of the nucleic acid. An "isolated" nucleic acid preferably has no sequences which naturally flank the nucleic acid in the genomic DNA of the organism from which the nucleic acid is derived (for example sequences which are located at the 5' and 3' ends of the nucleic acid). In various embodiments, the isolated Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase molecule can comprise for example fewer than approximately 5 kb, 4 kb, 3 kb, 2 kb, 1 kb, 0.5 kb or 0.1 kb of nucleotide sequences which naturally flank the nucleic acid molecule in the genomic DNA of the cell from which the nucleic acid is derived.

The nucleic acid molecules used in the process, for example a nucleic acid molecule with a nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69,

SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 or of a part thereof can be isolated using molecular-biological standard techniques and the sequence information provided herein. Also, for example a homologous sequence or homologous, conserved sequence regions can be identified at the DNA or amino acid level with the aid of comparative algorithms. They can be used as hybridization probe and standard hybridization techniques (such as, for example, those described in Sambrook et al., Molecular Cloning: A Laboratory Manual. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989) for isolating further nucleic acid sequences which can be used in the process. Moreover, a nucleic acid molecule comprising a complete sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 or a part thereof can be isolated by polymerase chain reaction, where oligonucleotide primers which are used on the basis of this sequence or parts thereof (for example a nucleic acid molecule comprising the complete sequence or part thereof can be isolated by polymerase chain reaction using oligonucleotide primers which have been generated based on this same sequence). For example, mRNA can be isolated from cells (for example by means of the guanidinium thiocyanate extraction method of Chirgwin et al. (1979) Biochemistry 18:5294-5299) and cDNA by means of reverse transcriptase (for example Moloney MLV reverse transcriptase, available from Gibco/BRL, Bethesda, MD, or AMV reverse transcriptase, available from Seikagaku America, Inc., St.Petersburg, FL). Synthetic oligonucleotide primers for the amplification by means of polymerase chain reaction can be generated based on one of the sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41,

SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 or with the aid of the amino acid sequences detailed in SEQ ID NO: 2, SEQ ID NO: 4, SEQ ID NO: 6, SEQ ID NO: 8, SEQ ID NO: 10, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16, SEQ ID NO: 18, SEQ ID NO: 20, SEQ ID NO: 22, SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 28, SEQ ID NO: 30, SEQ ID NO: 32, SEQ ID NO: 34, SEQ ID NO: 36, SEQ ID NO: 38, SEQ ID NO: 40, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 72, SEQ ID NO: 74, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 118, SEQ ID NO: 120, SEQ ID NO: 132, SEQ ID NO: 134, SEQ ID NO: 136, SEQ ID NO: 138 or SEQ ID NO: 184. A nucleic acid according to the invention can be amplified by standard PCR amplification techniques using cDNA or, alternatively, genomic DNA as template and suitable oligonucleotide primers. The nucleic acid amplified thus can be cloned into a suitable vector and characterized by means of DNA sequence analysis. Oligonucleotides which correspond to a desaturase nucleotide sequence can be generated by standard synthetic methods, for example using an automatic DNA synthesizer.

Homologs of the Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase nucleic acid sequences with the sequence SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117,

SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 means, for example, allelic variants with at least approximately 50 or 60%, preferably at least approximately 60 or 70%, more preferably at least approximately 70 or 80%, 90% or 95% and even more preferably at least approximately 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95 %, 96%, 97%, 98%, 99% or more identity or homology with a nucleotide sequence shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 or its homologs, derivatives or analogs or parts thereof. Furthermore, isolated nucleic acid molecules of a nucleotide sequence which hybridize with one of the nucleotide sequences shown in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 or with a part thereof, for example hybridized under stringent conditions. A part thereof is understood as meaning, in accordance with the invention, that at least 25 base pairs (= bp), 50 bp, 75 bp, 100 bp, 125 bp or 150 bp, preferably at least 175 bp, 200 bp, 225 bp, 250 bp, 275 bp or 300 bp, especially preferably 350 bp, 400 bp, 450 bp, 500 bp or more base pairs are used for the hybridization. It is also possible and advantageous to use the full sequence. Allelic variants comprise in particular functional variants which can be obtained by deletion, insertion or substitution of nucleotides from/into the sequence detailed in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23,

SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183, it being intended, however, that the enzyme activity of the resulting proteins which are synthesized is advantageously retained for the insertion of one or more genes. Proteins which retain the enzymatic activity of Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase or Δ4-desaturase, i.e. whose activity is essentially not reduced, means proteins with at least 10%, preferably 20%, especially preferably 30%, very especially preferably 40% of the original enzyme activity in comparison with the protein encoded by SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183. The homology was calculated over the entire amino acid or nucleic acid sequence region. The skilled worker has available a series of programs which are based on various algorithms for the comparison of various sequences. Here, the algorithms of Needleman and Wunsch or Smith and Waterman give particularly reliable results. The program PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5 1989: 151–153) or the programs Gap and BestFit [Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981)], which are part of the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)], were used for the sequence alignment. The sequence homology values which are indicated above as a percentage were determined over the entire sequence region using the program GAP and the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000. Unless otherwise specified, these settings were always used as standard settings for the

sequence alignments.

Homologs of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 means for example also bacterial, fungal and plant homologs, truncated sequences, single-stranded DNA or RNA of the coding and noncoding DNA sequence.

Homologs of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5, SEQ ID NO: 7, SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 17, SEQ ID NO: 19, SEQ ID NO: 21, SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 27, SEQ ID NO: 29, SEQ ID NO: 31, SEQ ID NO: 33, SEQ ID NO: 35, SEQ ID NO: 37, SEQ ID NO: 39, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 71, SEQ ID NO: 73, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 117, SEQ ID NO: 119, SEQ ID NO: 131, SEQ ID NO: 133, SEQ ID NO: 135, SEQ ID NO: 137 or SEQ ID NO: 183 also means derivatives such as, for example, promoter variants. The promoters upstream of the nucleotide sequences detailed can be modified by one or more nucleotide exchanges, by insertion(s) and/or deletion(s) without the functionality or activity of the promoters being adversely affected, however. It is furthermore possible that the modification of the promoter sequence enhances their activity or that they are replaced entirely by more active promoters, including those from heterologous organisms.

The abovementioned nucleic acids and protein molecules with Δ12-desaturase, ω3-desaturase, Δ9-elongase, Δ6-desaturase, Δ8-desaturase, Δ6-elongase, Δ5-desaturase, Δ5-elongase and/or Δ4-desaturase activity which are involved in the metabolism of lipids and fatty acids, PUFA cofactors and enzymes or in the transport of lipophilic compounds across membranes are used in the process according to the

invention for the modulation of the production of PUFAs in transgenic organisms, advantageously in plants, such as maize, wheat, rye, oats, triticale, rice, barley, soybean, peanut, cotton, Linum species such as linseed or flax, Brassica species such as oilseed rape, canola and turnip rape, pepper, sunflower, borage, evening primrose and Tagetes, Solanaceae plants such as potato, tobacco, eggplant and tomato, Vicia species, pea, cassava, alfalfa, bushy plants (coffee, cacao, tea), Salix species, trees (oil palm, coconut) and perennial grasses and fodder crops, either directly (for example when the overexpression or optimization of a fatty acid biosynthesis protein has a direct effect on the yield, production and/or production efficiency of the fatty acid from modified organisms) and/or can have an indirect effect which nevertheless leads to an enhanced yield, production and/or production efficiency of the PUFAs or a reduction of undesired compounds (for example when the modulation of the metabolism of lipids and fatty acids, cofactors and enzymes lead to modifications of the yield, production and/or production efficiency or the composition of the desired compounds within the cells, which, in turn, can affect the production of one or more fatty acids).

The combination of various precursor molecules and biosynthesis enzymes leads to the production of various fatty acid molecules, which has a decisive effect on lipid composition, since polyunsaturated fatty acids (= PUFAs) are not only incorporated into triacylglycerol but also into membrane lipids.

Brassicaceae, Boraginaceae, Primulaceae, or Linaceae are particularly suitable for the production of PUFAs, for example stearidonic acid, eicosapentaenoic acid and docosahexaenoic acid. Linseed (Linum usitatissimum) is especially advantageously suitable for the production of PUFAs with the nucleic acid sequences according to the invention, advantageously, as described, in combination with further desaturases and elongases.

Lipid synthesis can be divided into two sections: the synthesis of fatty acids and their binding to sn-glycerol-3-phosphate, and the addition or modification of a polar head group. Usual lipids which are used in membranes comprise phospholipids, glycolipids, sphingolipids and phosphoglycerides. Fatty acid synthesis starts with the conversion of acetyl-CoA into malonyl-CoA by acetyl-CoA carboxylase or into acetyl-ACP by acetyl transacylase. After condensation reaction, these two product molecules together form acetoacetyl-ACP, which is converted via a series of condensation, reduction and dehydratization reactions so that a saturated fatty acid molecule with the desired chain length is obtained. The production of the unsaturated fatty acids from these molecules is catalyzed by specific desaturases, either aerobically by means of molecular oxygen or anaerobically (regarding the fatty acid synthesis in microorganisms, see F.C. Neidhardt et al. (1996) E. coli and Salmonella. ASM Press: Washington, D.C., pp. 612-636 and references cited therein; Lengeler et al. (Ed.) (1999) Biology of Procaryotes. Thieme: Stuttgart, New York, and the references therein, and Magnuson, K., et al. (1993) Microbiological Reviews 57:522-542 and the references therein). To undergo the further elongation steps, the resulting phospholipid-bound fatty acids must be returned to the fatty acid CoA ester pool. This is made possible by acyl-

CoA:lysophospholipid acyltransferases. Moreover, these enzymes are capable of transferring the elongated fatty acids from the CoA esters back to the phospholipids. If appropriate, this reaction sequence can be followed repeatedly.

Examples of precursors for the biosynthesis of PUFAs are oleic acid, linoleic acid and linolenic acid. The C18-carbon fatty acids must be elongated to C20 and C22 in order to obtain fatty acids of the eicosa and docosa chain type. With the aid of the desaturases used in the process, such as the Δ12-, ω3-, Δ4-, Δ5-, Δ6- and Δ8-desaturases and/or Δ5-, Δ6-, Δ9-elongases, arachidonic acid, eicosapentaenoic acid, docosapentaenoic acid or docosahexaenoic acid, advantageously eicosapentaenoic acid and/or docosahexaenoic acid, can be produced and subsequently employed in various applications regarding foodstuffs, feedstuffs, cosmetics or pharmaceuticals. C_{20}- and/or C_{22}-fatty acids with at least two, advantageously at least three, four, five or six, double bonds in the fatty acid molecule, preferably C_{20}- or C_{22}-fatty acids with advantageously four, five or six double bonds in the fatty acid molecule, can be prepared using the abovementioned enzymes. Desaturation may take place before or after elongation of the fatty acid in question. This is why the products of the desaturase activities and the further desaturation and elongation steps which are possible result in preferred PUFAs with a higher degree of desaturation, including a further elongation from C_{20}- to C_{22}-fatty acids, to fatty acids such as γ-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, stearidonic acid, eicosatetraenoic acid or eicosapentaenoic acid. Substrates of the desaturases and elongases used in the process according to the invention are C_{16}-, C_{18}- or C_{20}-fatty acids such as, for example, linoleic acid, γ-linolenic acid, α-linolenic acid, dihomo-γ-linolenic acid, eicosatetraenoic acid or stearidonic acid. Preferred substrates are linoleic acid, γ-linolenic acid and/or α-linolenic acid, dihomo-γ-linolenic acid, arachidonic acid, eicosatetraenoic acid or eicosapentaenoic acid. The synthesized C_{20}- or C_{22}-fatty acids with at least two, three, four, five or six double bonds in the fatty acids are obtained in the process according to the invention in the form of the free fatty acid or in the form of their esters, for example in the form of their glycerides.

The term “glyceride” is understood as meaning glycerol esterified with one, two or three carboxyl radicals (mono-, di- or triglyceride). “Glyceride” is also understood as meaning a mixture of various glycerides. The glyceride or glyceride mixture may comprise further additions, for example free fatty acids, antioxidants, proteins, carbohydrates, vitamins and/or other substances.

For the purposes of the invention, a “glyceride” is furthermore understood as meaning glycerol derivatives. In addition to the above-described fatty acid glycerides, these also include glycerophospholipids and glyceroglycolipids. Preferred examples which may be mentioned in this context are the glycerophospholipids such as lecithin (phosphatidylcholine), cardiolipin, phosphatidylglycerol, phosphatidylserine and alkylacylglycerophospholipids.

Furthermore, fatty acids must subsequently be translocated to various modification

sites and incorporated into the triacylglycerol storage lipid. A further important step in lipid synthesis is the transfer of fatty acids to the polar head groups, for example by glycerol fatty acid acyltransferase (see Frentzen, 1998, Lipid, 100(4-5):161-166).

Publications on plant fatty acid biosynthesis and on the desaturation, the lipid metabolism and the membrane transport of lipidic compounds, on beta-oxidation, fatty acid modification and cofactors, triacylglycerol storage and triacylglycerol assembly, including the references therein, see the following papers: Kinney, 1997, Genetic Engeneering, Ed.: JK Setlow, 19:149-166; Ohlrogge and Browse, 1995, Plant Cell 7:957-970; Shanklin and Cahoon, 1998, Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:611-641; Voelker, 1996, Genetic Engeneering, Ed.: JK Setlow, 18:111-13; Gerhardt, 1992, Prog. Lipid R. 31:397-417; Gühnemann-Schäfer & Kindl, 1995, Biochim. Biophys Acta 1256:181-186; Kunau et al., 1995, Prog. Lipid Res. 34:267-342; Stymne et al., 1993, in: Biochemistry and Molecular Biology of Membrane and Storage Lipids of Plants, Ed.: Murata and Somerville, Rockville, American Society of Plant Physiologists, 150-158, Murphy & Ross 1998, Plant Journal. 13(1):1-16.

The PUFAs produced in the process comprise a group of molecules which higher animals are no longer capable of synthesizing and must therefore take up, or which higher animals are no longer capable of synthesizing themselves in sufficient quantity and must therefore take up additional quantities, although they can be synthesized readily by other organisms such as bacteria; for example, cats are no longer capable of synthesizing arachidonic acid.

Phospholipids for the purposes of the invention are understood as meaning phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol and/or phosphatidylinositol, advantageously phosphatidylcholine. The terms production or productivity are known in the art and comprise the concentration of the fermentation product (compounds of the formula I) which is formed within a specific period of time and in a specific fermentation volume (for example kg of product per hour per liter). It also comprises the productivity within a plant cell or a plant, that is to say the content of the desired fatty acids produced in the process relative to the content of all fatty acids in this cell or plant. The term production efficiency comprises the time required for obtaining a specific production quantity (for example the time required by the cell to establish a certain throughput rate of a fine chemical). The term yield or product/carbon yield is known in the art and comprises the efficiency of the conversion of the carbon source into the product (i.e. the fine chemical). This is usually expressed for example as kg of product per kg of carbon source. By increasing the yield or production of the compound, the amount of the molecules obtained of this compound, or of the suitable molecules of this compound obtained in a specific culture quantity over a specified period of time is increased. The terms biosynthesis or biosynthetic pathway are known in the art and comprise the synthesis of a compound, preferably an organic compound, by a cell from intermediates, for example in a multi-step and strongly regulated process. The terms catabolism or catabolic pathway are known in the art and comprise the cleavage of a compound, preferably of an organic

compound, by a cell to give catabolites (in more general terms, smaller or less complex molecules), for example in a multi-step and strongly regulated process. The term metabolism is known in the art and comprises the totality of the biochemical reactions which take place in an organism. The metabolism of a certain compound (for example the metabolism of a fatty acid) thus comprises the totality of the biosynthetic pathways, modification pathways and catabolic pathways of this compound in the cell which relate to this compound.

In a further embodiment, derivatives of the nucleic acid molecule according to the invention represented in SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183 encode proteins with at least 40%, advantageously approximately 50 or 60%, advantageously at least approximately 60 or 70% and more preferably at least approximately 70 or 80%, 80 to 90%, 90 to 95% and most preferably at least approximately 96%, 97%, 98%, 99% or more homology (= identity) with a complete amino acid sequence of SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 112, SEQ ID NO: 114, SEQ ID NO: 132, SEQ ID NO: 134 or SEQ ID NO: 184. The homology was calculated over the entire amino acid or nucleic acid sequence region. The program PileUp (J. Mol. Evolution., 25, 351-360, 1987, Higgins et al., CABIOS, 5 1989: 151–153) or the programs Gap and BestFit [Needleman and Wunsch (J. Mol. Biol. 48; 443-453 (1970) and Smith and Waterman (Adv. Appl. Math. 2; 482-489 (1981)], which are part of the GCG software packet [Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991)], were used for the sequence alignment. The sequence homology values which are indicated above as a percentage were determined over the entire sequence region using the program BestFit and the following settings: Gap Weight: 50, Length Weight: 3, Average Match: 10.000 and Average Mismatch: 0.000. Unless otherwise specified, these settings were always used as standard settings for the sequence alignments.

Moreover, the invention comprises nucleic acid molecules which differ from one of the nucleotide sequences shown in SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89,

SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183 (and parts thereof) owing to the degeneracy of the genetic code and which thus encode the same Δ12-desaturase, ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase, Δ6-elongase or Δ5-elongase as those encoded by the nucleotide sequences shown in SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183.

In addition to the Δ12-desaturases, ω3-desaturases, Δ5-elongases, Δ6-desaturases, Δ5-desaturases, Δ4-desaturases or Δ6-elongases shown in SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183, the skilled worker will recognize that DNA sequence polymorphisms which lead to changes in the amino acid sequences of the Δ12-desaturase, ω3-desaturase, Δ5-elongase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase and/or Δ6-elongase may exist within a population. These genetic polymorphisms in the Δ12-desaturase, ω3-desaturase, Δ5-elongase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase and/or Δ6-elongase gene may exist between individuals within a population owing to natural variation. These natural variants usually bring about a variance of 1 to 5% in the nucleotide sequence of the Δ12-desaturase, ω3-desaturase, Δ5-elongase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase and/or Δ6-elongase gene. Each and every one of these nucleotide variations and resulting amino acid polymorphisms in the Δ12-desaturase, ω3-desaturase, Δ5-elongase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase and/or Δ6-elongase which are the result of natural variation and do not modify the functional activity are to be encompassed by the invention.

Owing to their homology to the Δ12-desaturase, ω3-desaturase, Δ5-elongase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase and/or Δ6-elongase nucleic acids disclosed here, nucleic acid molecules which are advantageous for the process according to the invention can be isolated following standard hybridization techniques under stringent hybridization conditions, using the sequences or part thereof as hybridization probe. In this context it is possible, for example, to use isolated nucleic acid molecules which are least 15 nucleotides in length and which hybridize under

stringent conditions with the nucleic acid molecules which comprise a nucleotide sequence of SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183. Nucleic acids with at least 25, 50, 100, 200 or more nucleotides can also be used. The "hybridizes under stringent conditions" as used in the present context is intended to describe hybridization and washing conditions under which nucleotide sequences with at least 60% homology to one another usually remain hybridized with one another. Conditions are preferably such that sequences with at least approximately 65%, preferably at least approximately 70% and especially preferably at least 75% or more homology to one another usually remain hybridized to one another. These stringent conditions are known to the skilled worker and described, for example, in Current Protocols in Molecular Biology, John Wiley & Sons, N. Y. (1989), 6.3.1-6.3.6. A preferred nonlimiting example of stringent hybridization conditions is hybridizations in 6 x sodium chloride/sodium citrate (= SSC) at approximately 45°C, followed by one or more washing steps in 0.2 x SSC, 0.1% SDS at 50 to 65°C. The skilled worker knows that these hybridization conditions differ depending on the type of nucleic acid and, for example when organic solvents are present, regarding temperature and buffer concentration. Under "standard hybridization conditions", for example, the hybridization temperature is, depending on the type of nucleic acid, between 42°C and 58°C in aqueous buffer with a concentration of 0.1 to 5 x SSC (pH 7.2). If organic solvents, for example 50% formamide, are present in the abovementioned buffer, the temperature under standard conditions is approximately 42°C. The hybridization conditions for DNA:DNA hybrids, for example, are 0.1 x SSC and 20°C to 45°C, preferably 30°C to 45°C. The hybridization conditions for DNA:RNA hybrids are, for example, 0.1 x SSC and 30°C to 55°C, preferably 45°C to 55°C. The abovementioned hybridization conditions are determined by way of example for a nucleic acid with approximately 100 bp (= base pairs) in length and with a G + C content of 50% in the absence of formamide. The skilled worker knows how to determine the required hybridization conditions on the basis of the abovementioned textbooks or textbooks such as Sambrook et al., "Molecular Cloning", Cold Spring Harbor Laboratory, 1989; Hames and Higgins (Ed.) 1985, "Nucleic Acids Hybridization: A Practical Approach", IRL Press at Oxford University Press, Oxford; Brown (Ed.) 1991, "Essential Molecular Biology: A Practical Approach", IRL Press at Oxford University Press, Oxford.

In order to determine the percentage of homology (= identity) of two amino acid sequences (for example one of the sequences of SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88,

SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 114, SEQ ID NO: 132, SEQ ID NO: 134 or SEQ ID NO: 184) or of two nucleic acids (for example SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183) the sequences are written one under the other for an optimal comparison (for example, gaps may be introduced into the sequence of a protein or of a nucleic acid in order to generate an optimal alignment with the other protein or the other nucleic acid). Then, the amino acid residue or nucleotides at the corresponding amino acid positions or nucleotide positions are compared. If a position in a sequence is occupied by the same amino acid residue or the same nucleotide as the corresponding position in the other sequence, then the molecules are homologous at this position (i.e. amino acid or nucleic acid "homology" as used in the present context corresponds to amino acid or nucleic acid "identity"). The percentage of homology between the two sequences is a function of the number of positions which the sequences share (i.e. % homology = number of identical positions/total number of positions x 100). The terms homology and identity are therefore to be considered as synonymous.

An isolated nucleic acid molecule which encodes a Δ12-desaturase, ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase, Δ5-elongase and/or Δ6-elongase which is homologous to a protein sequence of SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 48, SEQ ID NO: 50, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, SEQ ID NO: 70, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 80, SEQ ID NO: 82, SEQ ID NO: 84, SEQ ID NO: 86, SEQ ID NO: 88, SEQ ID NO: 90, SEQ ID NO: 92, SEQ ID NO: 94, SEQ ID NO: 96, SEQ ID NO: 98, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 104, SEQ ID NO: 106, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 114, SEQ ID NO: 132, SEQ ID NO: 134 or SEQ ID NO: 184 can be generated by introducing one or more nucleotide substitutions, additions or deletions in/into a nucleotide sequence of SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183 so that one or more amino acid substitutions, additions or deletions are introduced in/into the protein which is encoded. Mutations in one of the sequences of SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47,

SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183 can be introduced by standard techniques such as site-specific mutagenesis and PCR-mediated mutagenesis. It is preferred to generate conservative amino acid substitutions in one or more of the predicted nonessential amino acid residues. In a "conservative amino acid substitution", the amino acid residue is replaced by an amino acid residue with a similar side chain. Families of amino acid residues with similar side chains have been defined in the art. These families comprise amino acids with basic side chains (for example lysine, arginine, histidine), acidic side chains (for example aspartic acid, glutamic acid), uncharged polar side chains (for example glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), unpolar side chains (for example alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (for example threonine, valine, isoleucine) and aromatic side chains (for example tyrosine, phenylalanine, tryptophan, histidine). A predicted nonessential amino acid residue in a Δ12-desaturase, ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase, Δ5-elongase or Δ6-elongase is thus preferably replaced by another amino acid residue from the same family of side chains. In another embodiment, the mutations can, alternatively, be introduced randomly over all or part of the sequence encoding the Δ12-desaturase, ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase, Δ5-elongase or Δ6-elongase, for example by saturation mutagenesis, and the resulting mutants can be screened by recombinant expression for the herein-described Δ12-desaturase, ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase, Δ5-elongase or Δ6-elongase activity in order to identify mutants which have retained the Δ12-desaturase, ω3-desaturase, Δ6-desaturase, Δ5-desaturase, Δ4-desaturase, Δ5-elongase or Δ6-elongase activity. Following the mutagenesis of one of the sequences SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81, SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183, the protein which is encoded can be expressed recombinantly, and the activity of the protein can be determined, for example using the tests described in the present text.

The invention furthermore relates to transgenic nonhuman organisms which comprise the nucleic acids SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 47, SEQ ID NO: 49, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 79, SEQ ID NO: 81,

SEQ ID NO: 83, SEQ ID NO: 85, SEQ ID NO: 87, SEQ ID NO: 89, SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 99, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 105, SEQ ID NO: 107, SEQ ID NO: 109, SEQ ID NO: 113, SEQ ID NO: 131, SEQ ID NO: 133 or SEQ ID NO: 183 according to the invention or a gene construct or a vector which comprise these nucleic acid sequences according to the invention. The nonhuman organism is advantageously a microorganism, a nonhuman animal or a plant, especially preferably a plant.

The present invention is illustrated in greater detail by the examples which follow, which are not to be construed as limiting. The content of all of the references, patent applications, patents and published patent applications cited in the present patent application is herewith incorporated by reference.

Examples:

Example 1: General cloning methods:

The cloning methods such as, for example, restriction cleavages, agarose gel electrophoresis, purification of DNA fragments, transfer of nucleic acids to nitrocellulose and nylon membranes, linkage of DNA fragments, transformation of E. coli cells, bacterial cultures and the sequence analysis of recombinant DNA were carried out as described by Sambrook et al. (1989) (Cold Spring Harbor Laboratory Press: ISBN 0-87969-309-6).

Example 2: Sequence analysis of recombinant DNA:

Recombinant DNA molecules were sequenced with an ABI laser fluorescence DNA sequencer by the method of Sanger (Sanger et al. (1977) Proc. Natl. Acad. Sci. USA74, 5463-5467). Fragments obtained by polymerase chain reaction were sequenced and verified to avoid polymerase errors in constructs to be expressed.

Example 3: Cloning of Oncorhynchus mykiss genes

The search for conserved regions in the protein sequences corresponding to the elongase genes detailed in the application identified two sequences with corresponding motifs in the Genbank sequence database.

Name of gene	Genbank No.	Amino acids
OmELO2	CA385234, CA364848, CA366480	264
OmELO3	CA360014, CA350786	295

Oncoryhnchus mykiss total RNA was isolated with the aid of the RNAeasy kit from Qiagen (Valencia, CA, US). Poly-A+ RNA (mRNA) was isolated from the total RNA with

the aid of oligo-dT-cellulose (Sambrook et al., 1989). The RNA was subjected to reverse transcription using the Reverse Transcription System kit from Promega, and the cDNA synthesized was cloned into the vector lambda ZAP (lambda ZAP Gold, Stratagene). The cDNA was then unpackaged in accordance with the manufacturer's instructions to give the plasmid DNA. The cDNA plasmid library was then used for the PCR for cloning expression plasmids.

Example 4: Cloning of expression plasmids for the purposes of heterologous expression in yeasts

The following oligonucleotides were used for the PCR reaction for cloning the two sequences for the heterologous expression in yeasts:

Primer	Nucleotide sequence
5' f* OmELO2	5' aagcttacataatggcttcaacatggcaa (SEQ ID NO: 179)
3' r* OmELO2	5' ggatccttatgtcttcttgctcttcctgtt (SEQ ID NO: 180)
5' f OmELO3	5' aagcttacataatggagacttttaat (SEQ ID NO: 181)
3' r OmELO3	5' ggatccttcagtcccccctcactttcc (SEQ ID NO: 182)

* f: forward, r: reverse

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
5.00 µl 10 x buffer (Advantage polymerase)+ 25 mM $MgCl_2$
5.00 µl 2 mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase
The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 minute at 55°C
Denaturation temperature: 1 minute at 94°C
Elongation temperature: 2 minutes at 72°C
Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzymes HindIII and BamHI. The yeast expression vector pYES3 (Invitrogen) was incubated in the same manner. Thereafter, the 812 bp PCR product, the 905 bp PCR product and the vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen gel purification kit following the manufacturer's instructions. Thereafter, vector and elongase cDNA were ligated. The Rapid Ligation kit from Roche was used for this purpose. The resulting plasmids pYES3-OmELO2 and pYES3-OmELO3 were verified by sequencing and

transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, pYES3 was transformed in parallel. Thereafter, the yeasts were plated onto minimal dropout tryptophan medium supplemented with 2% glucose. Cells which were capable of growth without tryptophan in the medium thus comprised the corresponding plasmids pYES3, pYES3-OmELO2 (SEQ ID NO: 51) and pYES3-OmELO3 (SEQ ID NO: 53). After the selection, in each each case two transformants were chosen for the further functional expression.

Example 5: Cloning expression plasmids for the purposes of seed-specific expression in plants

To transform plants, a further transformation vector based on pSUN-USP was generated. To this end, NotI cleavage sites were introduced at the 5' and 3' ends of the coding sequence, using the following primer pair:
PSUN-OmELO2
Forward: 5'-GCGGCCGCATAATGGCTTCAACATGGCAA (SEQ ID NO: 175)
Reverse: 3'-GCGGCCGCTTATGTCTTCTTGCTCTTCCTGTT (SEQ ID NO: 176)
PSUN-OMELO3
Forward: 5'-GCGGCCGCataatggagacttttaat (SEQ ID NO: 177)
Reverse: 3'-GCGGCCGCtcagtcccccctcactttcc (SEQ ID NO: 178)

Composition of the PCR mix (50 µl):
5.00 µl template cDNA
5.00 µl 10 x buffer (Advantage polymerase)+ 25 mM $MgCl_2$
5.00 µl 2 mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase
The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 minute at 55°C
Denaturation temperature: 1 minute at 94°C
Elongation temperature: 2 minutes at 72°C
Number of cycles: 35

The PCR products were incubated for 16 hours at 37°C with the restriction enzyme NotI. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the 7624 bp vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen gel purification kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation kit from Roche was used for this purpose. The resulting plasmids pSUN-OmELO2 and pSUN-OmELO3 were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter as EcoRI fragment into pSUN300. The polyadenylation signal is that of the octopine synthase gene from the A. tumefaciens Ti plasmid (ocs terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982). The USP promoter corresponds to the nucleotides 1-684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by means of commercially available T7 standard primers (Stratagene) and with the aid of a synthesized primer via a PCR reaction following standard methods (primer sequence: 5'–GTCGACCCGCGGACTAGTGGGCCCTCT-AGACCCGGGGGATCCGGATCTGCTGGCTATGAA–3', SEQ ID NO: 174). The PCR fragment was cut again with EcoRI/SalI and introduced into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

Example 6: Lipid extraction from yeasts and seeds

The effect of the genetic modification in plants, fungi, algae, ciliates or on the production of a desired compound (such as a fatty acid) can be determined by growing the modified microorganisms or the modified plant under suitable conditions (such as those described above) and analyzing the medium and/or the cellular components for the elevated production of desired product (i.e. of the lipids or a fatty acid). These analytical techniques are known to the skilled worker and comprise spectroscopy, thin-layer chromatography, various types of staining methods, enzymatic and microbiological methods and analytical chromatography such as high-performance liquid chromatography (see, for example, Ullman, Encyclopedia of Industrial Chemistry, Vol. A2, p. 89-90 and p. 443-613, VCH: Weinheim (1985); Fallon, A., et al., (1987) "Applications of HPLC in Biochemistry" in: Laboratory Techniques in Biochemistry and Molecular Biology, Vol. 17; Rehm et al. (1993) Biotechnology, Vol. 3, Chapter III: "Product recovery and purification", p. 469-714, VCH: Weinheim; Belter, P.A., et al. (1988) Bioseparations: downstream processing for Biotechnology, John Wiley and Sons; Kennedy, J.F., and Cabral, J.M.S. (1992) Recovery processes for biological Materials, John Wiley and Sons; Shaeiwitz, J.A., and Henry, J.D. (1988) Biochemical Separations, in: Ullmann's Encyclopedia of Industrial Chemistry, Vol. B3; Chapter 11, p. 1-27, VCH: Weinheim; and Dechow, F.J. (1989) Separation and purification techniques in biotechnology, Noyes Publications).

In addition to the abovementioned processes, plant lipids are extracted from plant material as described by Cahoon et al. (1999) Proc. Natl. Acad. Sci. USA 96 (22):12935-12940 and Browse et al. (1986) Analytic Biochemistry 152:141-145. The

qualitative and quantitative analysis of lipids or fatty acids is described by Christie, William W., Advances in Lipid Methodology, Ayr/Scotland: Oily Press (Oily Press Lipid Library; 2); Christie, William W., Gas Chromatography and Lipids. A Practical Guide - Ayr, Scotland: Oily Press, 1989, Repr. 1992, IX, 307 pp. (Oily Press Lipid Library; 1); "Progress in Lipid Research, Oxford: Pergamon Press, 1 (1952) - 16 (1977) under the title: Progress in the Chemistry of Fats and Other Lipids CODEN.

In addition to measuring the end product of the fermentation, it is also possible to analyze other components of the metabolic pathways which are used for the production of the desired compound, such as intermediates and by-products, in order to determine the overall production efficiency of the compound. The analytical methods comprise measuring the amount of nutrients in the medium (for example sugars, hydrocarbons, nitrogen sources, phosphate and other ions), measuring the biomass composition and the growth, analyzing the production of conventional metabolites of biosynthetic pathways and measuring gases which are generated during the fermentation. Standard methods for these measurements are described in Applied Microbial Physiology; A Practical Approach, P.M. Rhodes and P.F. Stanbury, Ed., IRL Press, p. 103-129; 131-163 and 165-192 (ISBN: 0199635773) and references cited therein.

One example is the analysis of fatty acids (abbreviations: FAME, fatty acid methyl ester; GC-MS, gas liquid chromatography/mass spectrometry; TAG, triacylglycerol; TLC, thin-layer chromatography).

The unambiguous detection for the presence of fatty acid products can be obtained by analyzing recombinant organisms using analytical standard methods: GC, GC-MS or TLC, as described on several occasions by Christie and the references therein (1997, in: Advances on Lipid Methodology, Fourth Edition: Christie, Oily Press, Dundee, 119-169; 1998, Gaschromatographie-Massenspektrometrie-Verfahren [Gas chromatography/mass spectrometric methods], Lipide 33:343-353).

The material to be analyzed can be disrupted by sonication, grinding in a glass mill, liquid nitrogen and grinding or via other applicable methods. After disruption, the material must be centrifuged. The sediment is resuspended in distilled water, heated for 10 minutes at 100°C, cooled on ice and recentrifuged, followed by extraction for one hour at 90°C in 0.5 M sulfuric acid in methanol with 2% dimethoxypropane, which leads to hydrolyzed oil and lipid compounds, which give transmethylated lipids. These fatty acid methyl esters are extracted in petroleum ether and finally subjected to a GC analysis using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 µm, 0.32 mm) at a temperature gradient of between 170°C and 240°C for 20 minutes and 5 minutes at 240°C. The identity of the resulting fatty acid methyl esters must be defined using standards which are available from commercial sources (i.e. Sigma).

Plant material is initially homogenized mechanically by comminuting in a pestle and mortar to make it more amenable to extraction.

This is followed by heating at 100°C for 10 minutes and, after cooling on ice, by resedimentation. The cell sediment is hydrolyzed for one hour at 90°C with 1 M methanolic sulfuric acid and 2% dimethoxypropane, and the lipids are transmethylated. The resulting fatty acid methyl esters (FAMEs) are extracted in petroleum ether. The extracted FAMEs are analyzed by gas liquid chromatography using a capillary column (Chrompack, WCOT Fused Silica, CP-Wax-52 CB, 25 m, 0.32 mm) and a temperature gradient of from 170°C to 240°C in 20 minutes and 5 minutes at 240°C. The identity of the fatty acid methyl esters is confirmed by comparison with corresponding FAME standards (Sigma). The identity and position of the double bond can be analyzed further by suitable chemical derivatization of the FAME mixtures, for example to give 4,4-dimethoxyoxazoline derivatives (Christie, 1998) by means of GC-MS.

Yeasts which had been transformed as described in Example 4 with the plasmids pYES3, pYES3-OmELO2 and pYES3-OmELO3 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 10 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Fatty acid methyl esters (FAMEs) were prepared from the yeast cell sediments by acid methananolysis. To this end, the cell sediments were incubated with 2 ml of 1N methanolic sulfuric acid and 2% (v/v) dimethoxypropane for 1 hour at 80°C. The FAMEs were extracted by extracting twice with petroleum ether (PE). To remove underivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph flame ionization detector. The condtiions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C with a rate of 5°C/min and finally 10 minutes at 250°C (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma).

The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 7: Functional characterization of OmELO2 and OmELO3:

While OmELO2 shows no elongase activity, OmELO3 was shown to have a pronounced activity with different substrates. The substrate specificity of OmElo3 was determined after expression and feeding a variety of fatty acids (figure 2). The substrates fed can be detected in large amounts in all transgenic yeasts. All transgenic yeasts show the synthesis of novel fatty acids, the products of the OmElo3 reaction. This means that functional expression of the gene OmElo3 has been possible.

Figure 2 shows that OmElo3 has a substrate specificity which leads with high specificity to the elongation of Δ5- and Δ6-fatty acids with one ω3-double bond. Moreover, ω6-fatty acids (C18 and C20) were furthermore also elongated with less specificity. The best substrates for OmElo3 (up to 66% elongation) were stearidonic acid (C18:4 ω3) and eicosapentaenoic acid (C20:5 ω3).

Example 8: Reconstitution of the synthesis of DHA in yeast

The reconstitution of the biosynthesis of DHA (22:6 ω3) was carried out starting from EPA (20:5 ω3) or stearidonic acid (18:4 ω3) by coexpressing OmElo3 together with the *Euglena gracilis* Δ4-desaturase or the *Phaeodactylum tricornutum* Δ5-desaturase and the *Euglena gracilis* Δ4-desaturase. To this end, the expression vectors pYes2-EgD4 and pESCLeu-PtD5 were additionally constructed. The abovementioned yeast strain which is already transformed with pYes3-OtElo3 (SEQ ID NO: 55), was transformed further with pYes2-EgD4, or simultaneously with pYes2-EgD4 and pESCLeu-PtD5. The transformed yeasts were selected on complete minimal dropout tryptophan and uracil medium agar plates supplemented with 2% glucose in the case of the pYes3-OmELO/pYes2-EgD4 strain and complete minimal dropout tryptophan, uracil and leucine medium in the case of the pYes3-OmELO/pYes2-EgD4+pESCLeu-PtD5 strain. Expression was induced by addition of 2% (w/v) galactose as indicated above. The cultures were incubated for a further 120 hours at 15°C.

Figure 3 shows the fatty acid profiles of transgenic yeasts which had been fed with 20:5 ω3. In the control yeast (A), which had been transformed with the vector pYes3-OmElo3 and the blank vector pYes2, 20:5 ω3 was elongated very efficiently to give 22:5 ω3 (65% elongation). The additional introduction of EgΔ-4-desaturase resulted in the conversion of 22:5 ω3 into 22:6 ω3 (DHA). The fatty acid composition of the transgenic yeasts is shown in Figure 5. After coexpression of OmElo3 and EgD4, up to 3% of DHA was detected in yeasts.

In a further coexpression experiment, OmElo3, EgD4 and a *P. tricornutum* Δ5-desaturase (PtD5) were expressed together. The transgenic yeasts were fed stearidonic acid (18:4 ω3) and they were analyzed (Figure 4). The fatty acid composition of these yeasts is shown in Figure 5. The fatty acid fed, 18:4 ω3, was elongated by OmElo3 to give 20:4 ω3 (60% elongation). The latter was desaturated by PtD5 to give 20:5 ω3. The PtD5 activity was 15%. Moreover, it was possible to elongate 20:5 ω3 by OmElo3 to give 22:5 ω3. Thereafter, the newly synthesized 22:5 ω3 was desaturated to 22:6 ω3 (DHA). Up to 0.7% of DHA was obtained in these experiments.

It can be seen from these experiments that the sequences OmElo3, EgD4 and PtD5 which are used in the present invention are suitable for the production of DHA in eukaryotic cells.

Example 9: Generation of transgenic plants

a) Generation of transgenic oilseed rape plants (modified method of Moloney et al., 1992, Plant Cell Reports, 8:238-242)

Binary vectors in Agrobacterium tumefaciens C58C1:pGV2260 or Escherichia coli (Deblaere et al, 1984, Nucl. Acids. Res. 13, 4777-4788) can be used for generating transgenic oilseed rape plants. To transform oilseed rape plants (Var. Drakkar, NPZ Nordeutsche Pflanzenzucht, Hohenlieth, Germany), a 1:50 dilution of an overnight culture of a positively transformed agrobacterial colony in Murashige-Skoog medium (Murashige and Skoog 1962 Physiol. Plant. 15, 473) supplemented with 3% sucrose (3MS medium) is used. Petiols or hypocotyls of freshly germinated sterile oilseed rape plants (in each case approx. 1 cm^2) are incubated with a 1:50 agrobacterial dilution for 5-10 minutes in a Petri dish. This is followed by 3 days of coincubation in the dark at 25°C on 3MS medium supplemented with 0.8% Bacto agar. The cultures are then grown for 3 days at 16 hours light/8 hours dark and the cultivation is continued in a weekly rhythm on MS medium supplemented with 500 mg/l Claforan (cefotaxim sodium), 50 mg/l kanamycin, 20 μM benzylaminopurine (BAP), now supplemented with 1.6 g/l of glucose. Growing shoots are transferred to MS medium supplemented with 2% sucrose, 250 mg/l Claforan and 0.8% Bacto agar. If no roots develop after three weeks, 2-indolebutyric acid was added to the medium as growth hormone for rooting.

Regenerated shoots were obtained on 2MS medium supplemented with kanamycin and Claforan; after rooting, they were transferred to compost and, after growing on for two weeks in a controlled-environment cabinet or in the greenhouse, allowed to flower, and mature seeds were harvested and analyzed by lipid analysis for elongase expression, such as Δ5-elongase or Δ6-elongase activity or ω3-desaturase activity. In this manner, lines with elevated contents of polyunsaturated C_{20}- and C_{22}-fatty acids can be identified.

b) Generation of transgenic linseed plants

Transgenic linseed plants can be generated for example by the method of Bell et al., 1999, In Vitro Cell. Dev. Biol.-Plant. 35(6):456-465 by means of particle bombardment. In general, linseed was transformed by an agrobacteria-mediated transformation, for example by the method of Mlynarova et al. (1994), Plant Cell Report 13: 282-285.

Example 10: Cloning Δ5-elongase genes from Thraustochytrium aureum ATCC34304 and Thraustochytrium ssp.

By comparing the various elongase protein sequences found in the present application, it was possible to define conserved nucleic acid regions (histidine box: His-Val-X-His-His, tyrosine box: Met-Tyr-X-Tyr-Tyr). An EST database of T. aureum ATCC34304 and Thraustochytrium ssp. was screened for further Δ5-elongases with the aid of these sequences. The following new sequences were found:

Name of gene	Nucleotides	Amino acids
BioTaurELO1	828 bp	275
TL16y2	831	276

T. aureum ATCC34304 and Thraustochytrium ssp. total RNA was isolated with the aid of the RNAeasy kit from Qiagen (Valencia, CA, US). mRNA was isolated from the total RNA with the aid of the PolyATract isolation system (Promega). The mRNA was subjected to reverse transcription using the Marathon cDNA amplification kit (BD Biosciences) and adaptors were ligated in accordance with the manufacturer's instructions. The cDNA library was then used for the PCR for cloning expression plasmids by means of 5'- and 3'-RACE (rapid amplification of cDNA ends).

Example 11: Cloning of expression plasmids for the heterologous expression in yeasts

The following oligonucleotides were used for the PCR reaction for cloning the sequence for the heterologous expression in yeasts:

Primer	Nucleotide sequence
5' f* BioTaurELO1	5' gacataatgacgagcaacatgag (SEQ ID NO: 170)
3' r* BioTaurELO1	5' cggcttaggccgacttggccttggg (SEQ ID NO: 171)
5'f*TL16y2	5' agacataatggacgtcgtcgagcagcaatg (SEQ ID NO: 172)
3'r*TL16y2	5' ttagatggtcttctgcttcttgggcgcc (SEQ ID NO: 173)

* f: forward, r: reverse

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
5.00 µl 10 x buffer (Advantage polymerase)+ 25 mM $MgCl_2$
5.00 µl 2 mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase
The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 minute at 55°C
Denaturation temperature: 1 minute at 94°C
Elongation temperature: 2 minutes at 72°C
Number of cycles: 35

The PCR products BioTaurELO1 (see SEQ ID NO: 65) and TL16y2 (see SEQ ID

NO: 83) were incubated for 30 minutes at 21°C together with the yeast expression vector pYES2.1-TOPO (Invitrogen) in accordance with the manufacturer's instructions. Here, the PCR product was ligated into the vector by means of a T-overhang and the activity of a topoisomerase (Invitrogen). After the incubation, E. coli DH5α cells were transformed. Suitable clones were identified by PCR, the plasmid DNA was isolated by means of the Qiagen DNAeasy kit and verified by sequencing. The correct sequence was then transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, the blank vector pYES2.1 was transformed in parallel. Thereafter, the yeasts were plated out on minimal dropout uracil medium supplemented with 2% glucose. Cells which were capable of growing in the medium without uracil thus comprise the corresponding plasmids pYES2.1, pYES2.1-BioTaurELO1 and pYES2.1-TL16y2. After the selection, in each case two transformants were chosen for the further functional expression.

Example 12: Cloning expression plasmids for the purposes of seed-specific expression in plants

To transform plants, a further transformation vector based on pSUN-USP was generated. To this end, NotI cleavage sites were introduced at the 5' and 3' ends of the coding sequence, using the following primer pair:

PSUN-BioTaurELO1
Forward: 5'-GCGGCCGCATAATGACGAGCAACATGAGC (SEQ ID NO: 166)
Reverse: 3'-GCGGCCGCTTAGGCCGACTTGGCCTTGGG (SEQ ID NO: 167)

PSUN-TL16y2
Forward: 5'-GCGGCCGCACCATGGACGTCGTCGAGCAGCAATG (SEQ ID NO: 168)
Reverse: 5'-GCGGCCGCTTAGATGGTCTTCTGCTTCTTGGGCGCC (SEQ ID NO: 169)

Composition of the PCR mix (50 µl):
5.00 µl template cDNA
5.00 µl 10 x buffer (Advantage polymerase)+ 25 mM $MgCl_2$
5.00 µl 2 mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase
The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 minute at 55°C
Denaturation temperature: 1 minute at 94°C
Elongation temperature: 2 minutes at 72°C
Number of cycles: 35

The PCR products were incubated for 16 hours at 37°C with the restriction enzyme NotI. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the 7624 bp vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen gel purification kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation kit from Roche was used for this purpose. The resulting plasmids pSUN-BioTaurELO1 and pSUN-TL16y2 were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter as EcoRI fragment into pSUN300. The polyadenylation signal is that of the octopine synthase gene from the A. tumefaciens Ti plasmid (ocs terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982). Der USP promoter corresponds to the nucleotides 1-684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by means of commercially available T7 standard primers (Stratagene) and with the aid of a synthesized primer via a PCR reaction following standard methods (primer sequence: 5'–GTCGACCCGCGGACTAGTGGGCCCTCT-AGACCCGGGGGATCCGGATCTGCTGGCTATGAA–3', SEQ ID NO: 165). The PCR fragment was cut again with EcoRI/SalI and introduced into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

The lipid extraction from yeasts and seeds was carried out as described in Example 6

Example 13: Functional characterization of BioTaurELO1 and TL16y2:

The substrate specificity of BioTaurELO1 was determined after expression and feeding of various fatty acids (figure 6). Figure 6 shows the feeding experiments for determining the functionality and substrate specificity with yeast strains which comprise either the vector pYes2.1 (control) or the vector pYes2.1-BioTaurELO1 (= BioTaur) with the Δ5-elongase. In both batches, 200 µM of γ-linolenic acid and eicosapentaenoic acid were added to the yeast incubation medium, and incubation was carried out for 24 hours. After the fatty acids had been extracted from the yeasts, they were transmethylated and separated by gas chromatography. The elongation products obtained from the two fatty acids which had been fed are identified by arrows.

The substrates fed can be detected in large amounts in all transgenic yeasts. All transgenic yeasts show the synthesis of novel fatty acids, the products of the

BioTaurELO1 reaction. This means that functional expression of the gene BioTaurELO1 has been possible.

Figure 6 shows that BioTaurELO1 shows a substrate specificity which leads with high specificity to the elongation of Δ5- and Δ6-fatty acids with one ω3-double bond. ω6-Fatty acids (C18 and C20) were furthermore also elongated. γ-Linolenic acid (C18:3 ω6) is converted at a rate of 65.28%, stearidonic acid (C18:4 ω3) at a rate of 65.66% and eicosapentaenoic acid (C20:5 ω3) at a rate of 22.01%. The substrate specificities of the various feeding experiments are shown in table 3 (see end of description).

The conversion rate of GLA when GLA and EPA were fed was 65.28%. The conversion rate of EPA when the same amounts of GLA and EPA were fed was 9.99%. If only EPA was fed, the EPA conversion rate was 22.01%. Arachidonic acid (= ARA) was also converted when fed. The conversion rate was 14.47%. Stearidonic acid (= SDA) was also converted. In this case, the conversion rate was 65.66%.

The functionality and substrate specificity of TL16y2 was determined after expression and feeding of various fatty acids. Table 4 shows the feeding experiments. The feeding experiments were carried out in the same manner as described for BioTaurELO1. The substrates fed can be detected in large amounts in all transgenic yeasts. All transgenic yeasts showed the synthesis of novel fatty acids, the products of the TL16y2 reaction (Fig. 11). This means that functional expression of the gene TL16y2 has been possible.

Table 4: Expression of TL16y2 in yeast.

Areas of the gas chromatographic analysis in %									
Plasmid	Fatty acid	C18:3 (n-6)	C18:4 (n-3)	C20:3 (n-6)	C20:4 (n-6)	C20:4 (n-3)	C20:5 (n-3)	C22:4 (n-6)	C22:5 (n-3)
pYES	250 uM EPA						13.79		
TL16y2	250 uM EPA						25.81		**2.25**
pYES	50 uM EPA						5.07		
TL16y2	50 uM EPA						2.48		**1.73**
pYES	250 uMGLA	8.31							
TL16y2	250 uM GLA	3.59		**10.71**					
pYES	250 uM ARA				16.03				
TL16y2	250 uM ARA				15.2		**3.87**		
pYES	250 uM SDA		26.79			0.35			
TL16y2	250 uM SDA		7.74			**29.17**			

The results for TL16y2 in comparison with the control, which are shown in Table 4, show the following conversion rates in percent: a) % conversion rate EPA (250 µM): 8%, b) % conversion rate EPA (50 µM): 41%, c) % conversion rate ARA: 20.3%, d) % conversion rate SDA: 79.4% and e) % conversion rate GLA: 74.9%.

Thus, TL16y2 shows Δ5-, Δ6- and Δ8-elongase activity. Among these, the activity for C18-fatty acids with Δ6-double bond is the highest. Depending on the concentration of fatty acids fed, this is followed by the elongation of C20-fatty acids with one Δ5- or Δ8-double bond.

Example 14: Cloning genes from Ostreococcus tauri

By searching for conserved regions in the protein sequences with the aid of the elongase genes listed in the application with Δ5-elongase activity or Δ6-elongase activity, it was possible to identify two sequences with corresponding motifs in an Ostreococcus tauri sequence database (genomic sequences). The sequences are the following

Name of gene	SEQ ID	Amino acids
OtELO1, (Δ5-elongase)	SEQ ID NO: 67	300
OtELO2, (Δ6-elongase)	SEQ ID NO: 69	292

OtElo1 has the highest similarity with a Danio rerio elongase (GenBank AAN77156; approx. 26% identity), while OtElo2 has the greatest similarity with the Physcomitrella Elo (PSE) [approx. 36% identity] (alignments were carried out using the tBLASTn algorithm (Altschul et al., J. Mol. Biol. 1990, 215: 403 – 410).

The cloning procedure was carried out as follows:

40 ml of an *Ostreococcus tauri* culture in the stationary phase were spun down and the pellet was resuspended in 100 µl of double-distilled water and stored at -20°C. The relevant genomic DNAs were amplified based on the PCR method. The corresponding primer pairs were selected in such a way that they contained the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) next to the start codon. The amplification of the OtElo-DNAs was carried out using in each case 1 µl of defrosted cells, 200 µM dNTPs, 2.5 U *Taq* polymerase and 100 pmol of each primer in a total volume of 50 µl. The conditions for the PCR were as follows: first denaturation at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a final elongation step at 72°C for 10 minutes.

Example 15: Cloning of expression plasmids for heterologous expression in yeasts

To characterize the function of the *Ostreococcus tauri* elongases, the open reading frames of the DNAs in question were cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), giving rise to pOTE1 and pOTE2.

The *Saccharomyces cerevisiae* strain 334 was transformed with the vector pOTE1 or pOTE2, respectively, by electroporation (1500 V). A yeast which was transformed with the blank vector pYES2 was used as control. The transformed yeasts were selected on complete minimal dropout uracil medium (CMdum) agar plates supplemented with 2% glucose. After the selection, in each case three transformants were selected for the further functional expression.

To express the Ot elongases, precultures consisting of in each case 5 ml of CMdum dropout uracil liquid medium supplemented with 2% (w/v) raffinose were initially inoculated with the selected transformants and incubated for 2 days at 30°C and 200 rpm. Then, 5 ml of CMdum (dropout uracil) liquid medium supplemented with 2% of raffinose and 300 µM various fatty acids were inoculated with the precultures to an OD_{600} of 0.05. Expression was induced by the addition of 2% (w/v) of galactose. The cultures were incubated for a further 96 hours at 20°C.

Example 16 Cloning of expression plasmids for the seed-specific expression in plants

To transform plants, a further transformation vector based on pSUN-USP was generated. To this end, NotI cleavage sites were inserted at the 5' and 3' end of the coding sequences, using PCR. The corresponding primer sequences were derived from the 5' and 3' regions of OtElo1 and OtElo2.

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
5.00 µl 10x buffer (Advantage polymerase)+ 25mM $MgCl_2$
5.00 µl 2mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed.
PCR reaction conditions:
Annealing temperature: 1 min 55°C
Denaturation temperature: 1 min 94°C
Elongation temperature: 2 min 72°C
Number of cycles: 35

The PCR products were incubated with the restriction enzyme NotI for 16 hours at 37°C. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR product and the vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids pSUN-OtELO1 and pSUN-OtELO2 were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz,P, Svab, Z, Maliga, P., (1994). The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the Ostreococcus gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene). Primer sequence: 5'–GTCGACCCGCGGACTAGTGGG-

CCCTCTAGACCCGGGGGATCCGGATCTGCTGGCTATGAA–3', SEQ ID NO: 164). The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

Example 17: Expression of OtELO1 and OtELO2 in yeasts

Yeasts which had been transformed with the plasmids pYES3, pYES3-OtELO1 and pYES3-OtELO2 as described in Example 15 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for one hour at 80°C together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C with a rate of 5°C/min and finally 10 min at 250°C (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 18: Functional characterization of OtELO1 and OtELO2:

The substrate specificity of OtElo1 was determined after expression and after feeding various fatty acids (Tab. 5). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the OtElo1 reaction. This means that the gene OtElo1 was expressed functionally.

Table 4 shows that OtElo1 has a narrow degree of substrate specificity. OtElo1 was only capable of elongating the C_{20}-fatty acids eicosapentaenoic acid (Figure 7) and arachidonic acid (Figure 8), but preferentially ω3-desaturated eicosapentaenoic acid.

Table 5:

Fatty acid substrate	Conversion rate (in %)
16:0	**-**
$16:1^{\Delta 9}$	**-**
18:0	**-**
$18:1^{\Delta 9}$	**-**
$18:1^{\Delta 11}$	**-**
$18:2^{\Delta 9,12}$	**-**
$18:3^{\Delta 6,9,12}$	**-**
$18:3^{\Delta 5,9,12}$	**-**
$20:3^{\Delta 8,11,14}$	**-**
$20:4^{\Delta 5,8,11,14}$	**10.8 ± 0.6**
$20:5^{\Delta 5,8,11,14,17}$	**46.8 ± 3.6**
$22:4^{\Delta 7,10,13,16}$	**-**
$22:6^{\Delta 4,7,10,13,16,19}$	**-**

Table 5 shows the substrate specificity of the elongase OtElo1 for C_{20}-polyunsaturated fatty acids with a double bond in the Δ5 position in comparison with various fatty acids.

The yeasts which had been transformed with the vector pOTE1 were grown in minimal medium in the presence of the fatty acids stated. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed by GLC. Each value represents the mean (n=3) ± standard deviation.

The substrate specificity of OtElo2 (SEQ ID NO: 81) was determined after expression and after feeding various fatty acids (Tab. 6). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the OtElo2 reaction. This means that the gene OtElo2 was expressed functionally.

Table 6:

Fatty acid substrate	Conversion rate (in %)
16:0	-
$16:1^{\Delta 9}$	-
$16:3^{\Delta 7,10,13}$	-
18:0	-
$18:1^{\Delta 6}$	-
$18:1^{\Delta 9}$	-
$18:1^{\Delta 11}$	-
$18:2^{\Delta 9,12}$	-
$18:3^{\Delta 6,9,12}$	15.3±
$18:3^{\Delta 5,9,12}$	-
$18:4^{\Delta 6,9,12,15}$	21.1±
$20:2^{\Delta 11,14}$	-
$20:3^{\Delta 8,11,14}$	-
$20:4^{\Delta 5,8,11,14}$	-
$20:5^{\Delta 5,8,11,14,17}$	-
$22:4^{\Delta 7,10,13,16}$	-
$22:5^{\Delta 7,10,13,16,19}$	-
$22:6^{\Delta 4,7,10,13,16,19}$	-

Table 6 shows the substrate specificity of the elongase OtElo2 with regard to various fatty acids.

The yeasts which had been transformed with the vector pOTE2 were grown in minimal medium in the presence of the fatty acids stated. The fatty acid methyl esters were synthesized by subjecting inact cells to acid methanolysis. Thereafter, the FAMEs were analyzed by GLC. Each value represents the mean (n=3) ± standard deviation.

The enzymatic activity shown in Table 3 clearly demonstrates that OtElo2 is a Δ6-elongase.

Example 19: Cloning of genes from Thalassiosira pseudonana

By searching for conserved regions in the protein sequences with the aid of the elongase genes with Δ5-elongase activity or Δ6-elongase activity, which are detailed in

the application, it is possible to identify two sequences with corresponding motifs in a Thalassiosira pseudonana sequence database (genomic sequences). The sequences were the following:

Name of gene	SEQ ID	Amino acids
TpELO1 (Δ5-elongase)	43	358
TpELO2 (Δ5-elongase)	59	358
TpELO3 (Δ6-elongase)	45	272

A 2 l culture of T. pseudonana was grown in f/2 medium (Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. In *Culture of Marine Invertebrate Animals* (Eds. Smith, W.L. and Chanley, M.H.), Plenum Press, New York, pp 29–60) for 14 days at a light intensity of 80 E/cm^2. After centrifugation of the cells, RNA was isolated with the aid of the RNAeasy kits from Qiagen (Valencia, CA, US) following the manufacturer's instructions. The mRNA was subjected to reverse transcription with the Marathon cDNA amplification kit (BD Biosciences), and adaptors were ligated in accordance with the manufacturer's instructions. The cDNA library was then used for the PCR for cloning expression plasmids by means of 5'- and 3'-RACE (rapid amplification of cDNA ends).

Example 20: Cloning of expression plasmids for the purposes of heterologous expression in yeasts

The primer pairs in question were selected in such a way that they bore the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) next to the start codon. The amplification of the TpElo DNAs was carried out in each case with 1 µl cDNA, 200 µM dNTPs, 2.5 U *Advantage* polymerase and 100 pmol of each primer in a total volume of 50 µl. The PCR conditions were as follows: first denaturation at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a last elongation step at 72°C for 10 minutes.

The following oligonucleotides for the PCR reaction were used for cloning the sequence for the heterologous expression in yeasts:

Name of gene, and SEQ ID NO:	Primer sequence
TpELO1 (Δ5-elongase), SEQ ID NO: 59	F:5'-accatgtgctcaccaccgccgtc (SEQ ID NO: 158) R:5'- ctacatggcaccagtaac (SEQ ID NO: 159)
TpELO2 (Δ5-elongase), SEQ ID NO: 85	F:5'-accatgtgctcatcaccgccgtc (SEQ ID NO: 160) R:5'-ctacatggcaccagtaac (SEQ ID NO: 161)
TpELO3 (Δ6-elongase), SEQ ID NO: 45	F:5'-accatggacgcctacaacgctgc (SEQ ID NO: 162) R:5'- ctaagcactcttcttcttt (SEQ ID NO: 163)

*F = forward primer, R = reverse primer

The PCR products were incubated for 30 minutes at 21°C with the yeast expression vector pYES2.1-TOPO (Invitrogen) following the manufacturer's instructions. The PCR product was ligated into the vector by means of a T-overhang and the activity of a topoisomerase (Invitrogen). After the incubation, E. coli DH5α cells were transformed. Suitable clones were identified by PCR, the plasmid DNA was isolated by means of the Qiagen DNAeasy kit and verified by sequencing. The correct sequence was then transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, the blank vector pYES2.1 was transformed in parallel. Thereafter, the yeasts were plated out on minimal dropout uracil medium supplemented with 2% glucose. Cells which were capable of growing in the medium without uracil thus comprise the corresponding plasmids pYES2.1, pYES2.1-TpELO1, pYES2.1-ELO2 and pYES2.1-TpELO3. After the selection, in each case two transformants were chosen for the further functional expression.

Example 21: Cloning expression plasmids for the purposes of seed-specific expression in plants

To transform plants, a further transformation vector based on pSUN-USP is generated. To this end, NotI cleavage sites are introduced at the 5' and 3' ends of the coding sequence, using the following primer pair:

PSUN-TPELO1
Forward: 5'-GCGGCCGCACCATGTGCTCACCACCGCCGTC (SEQ ID NO: 152)
Reverse: 3'-GCGGCCGCCTACATGGCACCAGTAAC (SEQ ID NO: 153)

PSUN-TPELO2
Forward: 5'-GCGGCCGCACCATGTGCTCATCACCGCCGTC (SEQ ID NO: 154)

Reverse: 3'-GCGGCCGCCTACATGGCACCAGTAAC (SEQ ID NO: 155)

PSUN-TPELO3
Forward: 5'-GCGGCCGCaccatggacgcctacaacgctgc (SEQ ID NO: 156)
Reverse: 3'-GCGGCCGCCTAAGCACTCTTCTTCTTT (SEQ ID NO: 157)

Composition of the PCR mix (50 µl):
5.00 µl template cDNA
5.00 µl 10 x buffer (Advantage polymerase)+ 25 mM $MgCl_2$
5.00 µl 2 mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 minute at 55°C
Denaturation temperature: 1 minute at 94°C
Elongation temperature: 2 minutes at 72°C
Number of cycles: 35

The PCR products were incubated for 16 hours at 37°C with the restriction enzyme NotI. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the 7624 bp vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen gel purification kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation kit from Roche was used for this purpose. The resulting plasmids pSUN-TPELO1, pSUN-TPELO2 and pSUN-TPELO3 were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter as EcoRI fragment into pSUN300. The polyadenylation signal is that of the octopine synthase gene from the A. tumefaciens Ti plasmid (ocs terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982). The USP promoter corresponds to the nucleotides 1-684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by means of commercially available T7 standard primers (Stratagene) and with the aid of a synthesized primer via a PCR reaction following standard methods.

(Primer sequence: 5'–GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGA-TCCGGATCTGCTGGCTATGAA–3'; SEQ ID NO: 151).

The PCR fragment was cut again with EcoRI/SalI and introduced into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

The lipid extraction from yeasts and seeds was carried out as described in Example 6

Example 22: Expression of TpELO1, TpELO2 and TpELO3 in yeasts

Yeasts which had been transformed with the plasmids pYES2, pYES2-TpELO1, pYES2-TpELO2 and pYES2-TpELO3 as described in Example 4 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for 1 hour at 80°C together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C with a rate of 5°C/min and finally 10 min at 250°C (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 23: Functional characterization of TpELO1 and TPELO3:

The substrate specificity of TpElo1 was determined after expression and after feeding various fatty acids (Fig. 9). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the TpElo1 reaction. This means that the gene TpElo1 was expressed functionally.

Table 7 shows that TpElo1 has a narrow degree of substrate specificity. TpElo1 was

only capable of elongating the C20-fatty acids eicosapentaenoic acid and arachidonic acid, but preferentially ω3-desaturated eicosapentaenoic acid.

The yeasts which had been transformed with the vector pYES2-TpELO1 were grown in minimal medium in the presence of the fatty acids stated. Then, the fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter the FAMEs were analyzed by GLC.

Table 7: Expression of TpELO1 in yeast. Columns 1 and 3 show the control reactions for columns 2 (fed 250 μM 20:4 Δ5,8,11,14) and 4 (fed 250 μM 20:5 Δ5,8,11,14,17).

	Expression	Expression	Expression	Expression
Fatty acids	1	2	3	4
16:0	18.8	17.8	25.4	25.2
$16:1^{\Delta 9}$	28.0	29.8	36.6	36.6
18:0	5.2	5.0	6.8	6.9
$18:1^{\Delta 9}$	25.5	23.6	24.6	23.9
$20:4^{\Delta 5,8,11,14}$	**22.5**	**23.4**	-	-
$22:4^{\Delta 7,10,13,16}$	**-**	**0.4**	-	-
$20:5^{\Delta 5,8,11,14,17}$	-	-	**6.6**	**6.5**
$22:5^{\Delta 7,10,13,16,19}$	-	-	-	**0.9**
% conversion rate	**0**	**1.7**	**0**	**12.2**

The substrate specificity of TpElo3 was determined after expression and after feeding various fatty acids (Fig. 10). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the TpElo3 reaction. This means that the gene TpElo3 was expressed functionally.

Table 8 shows that TpElo3 has narrow substrate specificity. TpElo3 was only capable of elongating the C18-fatty acids γ-linolenic acid and stearidonic acid, but preferred ω3-desaturated stearidonic acid.

The yeasts which had been transformed with the vector pYES2-TpELO3 were grown in minimal medium in the presence of the fatty acids stated. Then, the fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter the FAMEs were analyzed by GLC.

Table 8: Expression von TpELO3 in yeast. Column 1 shows the fatty acid profile of yeast without feeding. Column 2 shows the control reaction. In columns 3 to 6, γ-linolenic acid, stearidonic acid, arachidonic acid and eicosapentaenoic acid were fed (250 μM of each fatty acid).

Fatty acids	1	2	3	4	5	6
16:0	17.9	20.6	17.8	16.7	18.8	18.8
$16:1^{\Delta 9}$	41.7	18.7	27.0	33.2	24.0	31.3
18:0	7.0	7.7	6.4	6.6	5.2	6,0
$18:1^{\Delta 9}$	33.3	16.8	24.2	31.8	25.5	26.4
$18:2^{\Delta 9,12}$	-	**36.1**	-	-	-	-
$18:3^{\Delta 6,9,12}$	-	-	**6.1**	-	-	
$18:4^{\Delta 6,9,12,15}$	-	-	-	**1.7**	-	
$20:2^{\Delta 11,14}$	-	**0**	-	-	-	
$20:3^{\Delta 8,11,14}$	-	-	**18.5**	-	-	
$20:4^{\Delta 8,11,14,17}$	-	-	-	**10.0**	-	
$20:4^{\Delta 5,8,11,14}$	-	-	-	-	**22.5**	
$22:4^{\Delta 7,10,13,16}$	-	-	-	-	**0**	
$20:5^{\Delta 5,8,11,14,17}$	-	-	-	-	-	**17.4**
$22:5^{\Delta 7,10,13,16,19}$	-	-	-	-	-	**0**
% conversion rate	**0**	**0**	**75**	**85**	**0**	**0**

Example 24: Cloning an expression plasmid for heterologous expression of Pi-omega3Des in yeasts

For heterologous expression in yeasts, the Pi-omega3Des clone was cloned into the yeast expression vector pYES3 via PCR with suitable Pi-omega3Des-specific primers. Only the open reading frame, of the gene, which encoded the Pi-omega3Des protein was amplified; it was provided with two cleavage sites for cloning into the expression vector pYES3:

Forward Primer: 5'-TAAGCTTACATGGCGACGAAGGAGG (SEQ ID NO: 149)
Reverse Primer: 5'-TGGATCCACTTACGTGGACTTGGT (SEQ ID NO: 150)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
5.00 µl 10 x buffer (Advantage polymerase)+ 25 mM $MgCl_2$
5.00 µl 2 mM dNTP
1.25 µl of each primer (10 pmol/µl of the 5'-ATG and of the 3'-stop primer)
0.50 µl Advantage polymerase
The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 minute at 55°C
Denaturation temperature: 1 minute at 94°C

Elongation temperature: 2 minutes at 72°C
Number of cycles: 35

The PCR product was incubated for 2 hours at 37°C with the restriction enzymes HindIII and BamHI. The yeast expression vector pYES3 (Invitrogen) was incubated in the same manner. Thereafter, the 1104 bp PCR product and the vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen gel purification kit following the manufacturer's instructions. Thereafter, vector and desaturase cDNA were ligated. The Rapid Ligation kit from Roche was used for this purpose. The resulting plasmid pYES3-Pi-omega3Des were verified by sequencing and transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, pYES3 was transformed in parallel. Thereafter, the yeasts were plated onto minimal dropout tryptophan medium supplemented with 2% glucose. Cells which were capable of growth without tryptophan in the medium thus comprised the corresponding plasmids pYES3, pYES3-Pi-omega3Des. After the selection, in each case two transformants were chosen for the further functional expression.

Example 25: Cloning expression plasmids for the purposes of seed-specific expression in plants

To transform plants, a further transformation vector based on pSUN-USP was generated. To this end, NotI cleavage sites were introduced at the 5' and 3' ends of the coding sequence, using the following primer pair:
PSUN-Pi-omega3Des

Reverse: 3'-GCGGCCGCTTACGTGGACTTGGTC (SEQ ID NO: 147)
Forward: 5'-GCGGCCGCatGGCGACGAAGGAGG (SEQ ID NO: 148)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
5.00 µl 10 x buffer (Advantage polymerase)+ 25 mM $MgCl_2$
5.00 µl 2 mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase
The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 minute at 55°C
Denaturation temperature: 1 minute at 94°C
Elongation temperature: 2 minutes at 72°C
Number of cycles: 35

The PCR products were incubated for 4 hours at 37°C with the restriction enzyme NotI.

The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the 7624 bp vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of Qiagen gel purification kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation kit from Roche was used for this purpose. The resulting plasmid pSUN-Piomega3Des was verified by sequencing.

Example 26: Expression of Pi-omega3Des in yeasts

Yeasts which had been transformed with the plasmid pYES3 or pYES3-Pi-omega3Des as described in Example 24 were analyzed as follows:
The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Fatty acid methyl esters (FAMEs) were prepared from the yeast cell sediments by acid methanolysis. To this end, the cell sediments were incubated with 2 ml of 1N methanolic sulfuric acid and 2% (v/v) dimethoxypropane for 1 hour at 80°C. The FAMEs were extracted by extracting twice with petroleum ether (PE). To remove underivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C with a rate of 5°C/min and finally 10 minutes at 250°C (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 27: Functional characterization of Pi-omega3Des:

The substrate specificity of Pi-omega3Des was determined after expression and after feeding various fatty acids (Figure 12 to 18). The substrates fed can be detected in large amounts in all of the transgenic yeasts, proving the uptake of these fatty acids into the yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the Pi-omega3Des reaction. This means that the gene Pi-omega3Des was expressed functionally

Figure 12 shows the desaturation of linoleic acid (18:2 ω-6-fatty acid) to α-linolenic acid (18:3 ω-3-fatty acid) by Pi-omega3Des. The fatty acid methyl esters were synthesized by subjecting intact cells which had been transformed with the blank vector pYES2

(Figure 12 A) or the vector pYes3-Pi-omega3Des (Figure 12 B) to acid methanolysis. The yeasts were grown in minimal medium in the presence of C18:$2^{\Delta 9,12}$-fatty acid (300 μM). Thereafter, the FAMEs were analyzed via GLC.

Figure 13 shows the desaturation of γ-linolenic acid (18:3 ω-6-fatty acid) to stearidonic acid (18:4 ω-3-fatty acid) by Pi-omega3Des.
The fatty acid methyl esters were synthesized by subjecting intact cells which had been transformed with the blank vector pYES2 (Figure 13 A) or the vector pYes3-Pi-omega3Des (Figure 13 B) to acid methanolysis. The yeasts were grown in minimal medium in the presence of γ-C18:$3^{\Delta 6,9,12}$-fatty acid (300 μM). Thereafter, the FAMEs were analyzed via GLC.

Figure 14 shows the desaturation of C20:2-ω-6-fatty acid to C20:3-ω-3-fatty acid by Pi-omega3Des. The fatty acid methyl esters were synthesized by subjecting intact cells which had been transformed with the blank vector pYES2 (Figure 14 A) or the vector pYes3-Pi-omega3Des (Figure 14 B) to acid methanolysis. The yeasts were grown in minimal medium in the presence of C20:$2^{\Delta 11,14}$-fatty acid (300 μM). Thereafter, the FAMEs were analyzed via GLC.

Figure 15 shows the desaturation of C20:3-ω-6-fatty acid to C20:4-ω-3-fatty acid by Pi-omega3Des. The fatty acid methyl esters were synthesized by subjecting intact cells which had been transformed with the blank vector pYES2 (Figure 15 A) or the vector pYes3-Pi-omega3Des (Figure 15 B) to acid methanolysis. The yeasts were grown in minimal medium in the presence of C20:$3^{\Delta 8,11,14}$-fatty acid (300 μM). Thereafter, the FAMEs were analyzed via GLC.

Figure 16 shows the desaturation of arachidonic acid (C20:4-ω-6-fatty acid) to eicosapentaenoic acid (C20:5-ω-3-fatty acid) by Pi-omega3Des.

The fatty acid methyl esters were synthesized by subjecting intact cells which had been transformed with the blank vector pYES2 (Figure 16 A) or the vector pYes3-Pi-omega3Des (Figure 16 B) to acid methanolysis. The yeasts were grown in minimal medium in the presence of C20:$4^{\Delta 5,8,11,14}$-fatty acid (300 μM). Thereafter, the FAMEs were analyzed via GLC.

Figure 17 shows the desaturation of docosatetraenoic acid (C22:4-ω-6-fatty acid) to docosapentaenoic acid (C22:5-ω-3-fatty acid) by Pi-omega3Des. The fatty acid methyl esters were synthesized by subjecting intact cells which had been transformed with the blank vector pYES2 (Figure 17 A) or the vector pYes3-Pi-omega3Des (Figure 17 B) to acid methanolysis. The yeasts were grown in minimal medium in the presence of C22:$4^{\Delta 7,10,13,16}$-fatty acid (300 μM). Thereafter, the FAMEs were analyzed via GLC.

The substrate specificity of Pi-omega3Des toward various fatty acids can be seen from Figure 18. The yeasts which had been transformed with the vector pYes3-Pi-omega3Des were grown in minimal medium in the presence of the fatty acids stated.

The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC. Each value represents a mean of three measurements. The conversion rates (% desaturation) were calculated using the formula:
[product]/[product]+[substrate]*100.

As described in Example 9, Pi-omega3Des can also be used for generating transgenic plants. The lipids can then be extracted from the seeds of these plants as described in Example 6.

Example 28: Cloning desaturase genes from Ostreococcus tauri

The search for conserved regions in the protein sequences with the aid of conserved motifs (His boxes, Domergue et al. 2002, Eur. J. Biochem. 269, 4105-4113) allowed the identification of five sequences with corresponding motifs in an Ostreococcus tauri sequence database (genomic sequences). The sequences are the following:

Name of gene	SEQ ID	Amino acids	Homology
OtD4	SEQ ID NO: 95	536	Δ4-desaturase
OtD5.1	SEQ ID NO: 91	201	Δ5-desaturase
OtD5.2	SEQ ID NO: 93	237	Δ5-desaturase
OtD6.1	SEQ ID NO: 89	457	Δ6-desaturase
OtFad2	SEQ ID NO: 107	361	Δ12-desaturase

The alignments for finding homologies of the individual genes were carried out using the tBLASTn algorithm (Altschul et al., J. Mol. Biol. 1990, 215: 403-410).

Cloning was carried out as follows:

40 ml of an *Ostreococcus tauri* culture in the stationary phase were spun down and the pellet was resuspended in 100 µl of double-distilled water and stored at -20°C. The relevant genomic DNAs were amplified based on the PCR method. The corresponding primer pairs were selected in such a way that they contained the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) next to the start codon. The amplification of the OtDes-DNAs was carried out using in each case 1 µl of defrosted cells, 200 µM dNTPs, 2.5 U *Taq* polymerase and 100 pmol of each primer in a total volume of 50 µl. The conditions for the PCR were as follows: first denaturation at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a final elongation step at 72°C for 10 minutes.

The following primers were employed for the PCR:

OtDes6.1 Forward: 5'ggtaccacataatgtgcgtggagacggaaaataacg3' (SEQ ID NO: 145)

OtDes6.1 Reverse: 5'ctcgagttacgccgtctttccggagtgttggcc3' (SEQ ID NO: 146)

Example 29: Cloning of expression plasmids for heterologous expression in yeasts

To characterize the function of the desaturase OtDes6.1 (= Δ6-desaturase) from *Ostreococcus tauri*, the open reading frame of the DNA upstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen) was cloned, giving rise to the corresponding pYES2.1-OtDes6.1 clone. In a similar manner, further Ostreococcus desaturate genes can be cloned.

The *Saccharomyces cerevisiae* strain 334 was transformed with the vector pYES2.1-OtDes6.1 by electroporation (1500 V). A yeast which was transformed with the blank vector pYES2 was used as control. The transformed yeasts were selected on complete minimal dropout uracil medium (CMdum) agar plates supplemented with 2% glucose. After the selection, in each case three transformants were selected for the further functional expression.

To express the OtDes6.1 desaturase, precultures consisting of in each case 5 ml of CMdum dropout uracil liquid medium supplemented with 2% (w/v) raffinose were initially inoculated with the selected transformants and incubated for 2 days at 30°C and 200 rpm. Then, 5 ml of CMdum (dropout uracil) liquid medium supplemented with 2% of raffinose and 300 µM various fatty acids were inoculated with the precultures to an OD_{600} of 0.05. Expression was induced by the addition of 2% (w/v) of galactose. The cultures were incubated for a further 96 hours at 20°C.

Example 30: Cloning of expression plasmids for the seed-specific expression in plants

A further transformation vector based on pSUN-USP is generated for the transformation of plants. To this end, NotI cleavage sites are introduced at the 5' and 3' ends of the coding sequences, using PCR. The corresponding primer sequences are derived from the 5' and 3 regions of the desaturases.

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
5.00 µl 10x buffer (Advantage polymerase)+ 25mM $MgCl_2$
5.00 µl 2mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 min 55°C
Denaturation temperature: 1 min 94°C
Elongation temperature: 2 min 72°C
Number of cycles: 35

The PCR products were incubated with the restriction enzyme NotI for 16 hours at 37°C. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz,P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the Ostreococcus gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve,H., Dhaese,P., Seurinck,J., Lemmers,M., Van Montagu,M. and Schell,J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene). (Primer sequence:
5'–GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCC
GGATCTGCTGGCTATGAA–3', SEQ ID NO: 144).
The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

Example 31: Expression of OtDes6.1 in yeasts

Yeasts which had been transformed with the plasmids pYES2 and pYES2-OtDes6.2 as described in Example 4 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for one hour at 80°C together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm,

Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C with a rate of 5°C/min and finally 10 min at 250°C (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 32: Functional characterization of Ostreococcus desaturases:

The substrate specificity of desaturases can be determined after expression in yeast (see Examples cloning of desaturase genes, yeast expression) by feeding, using various yeasts. Descriptions for the determination of the individual activities can be found in WO 93/11245 for Δ15–desaturases, WO 94/11516 for Δ12–desaturases, WO 93/06712, US 5,614,393, US 5614393, WO 96/21022, WO 0021557 and WO 99/27111 for Δ6-desaturases, Qiu et al. 2001, J. Biol. Chem. 276, 31561-31566 for Δ4-desaturases, Hong et al. 2002, Lipids 37,863-868 for Δ5-desaturases.

Table 9 shows the substrate specificity of the desaturase OtDes6.1 with regard to various fatty acids. The substrate specificity of OtDes6.1 was determined after expression and after feeding various fatty acids. The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the OtDes6.1 reaction (Fig. 20). This means that the gene OtDes6.1 was expressed functionally.

The yeasts which had been transformed with the vector pYES2-OtDes6.1 were grown in minimal medium in the presence of the stated fatty acids. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC. Each value represents the mean (n=3) ± standard deviation. The activity corresponds to the conversion rate calculated using the formula [substrate/(substrate+product)*100].

Table 9 shows that OtDes6.1 has substrate specificity for linoleic and linolenic acid (18:2 and 18:3), since these fatty acids result in the highest acitvities. In contrast, the activity for oleic acid (18:1) and palmitoleic acid (16:1) is markedly less pronounced. The preferred conversion of linoleic and linolenic acid demonstrates the suitability of this desaturase for the production of polyunsaturated fatty acids.

Substrates	Activity in %
$16:1^{\Delta 9}$	5.6
$18:1^{\Delta 9}$	13.1
$18:2^{\Delta 9,12}$	68.7
$18:3^{\Delta 9,12,15}$	64.6

Figure 20 shows the conversion of linoleic acid by OtDes6.1. The FAMEs were analyzed via gas chromatography. The substrate fed (C18:2) is converted into γ-C18:3. Both starting material and product formed are indicated by arrows.

Figure 21 shows the conversion of linoleic acid (= LA) and α-linolenic acid (= ALA) in the presence of OtDes6.1 to give γ-linolenic acid (= GLA) and stearidonic acid (= STA), respectively (Figures 21 A and C). Furthermore, Figure 21 shows the conversion of linoleic acid (= LA) and α-linolenic acid (= ALA) in the presence of the Δ6-desaturase OtDes6.1 together with the Δ6-elongase PSE1 from Physcomitrella patens (Zank et al. 2002, Plant J. 31:255-268) and the Δ5-desaturase PtD5 from Phaeodactylum tricornutum (Domergue et al. 2002, Eur. J. Biochem. 269, 4105-4113) to give dihomo-γ-linolenic acid (= DHGLA) and arachidonic acid (= ARA, Figure 21 B) and to give dihomostearidonic acid (= DHSTA) and eicosapentaenoic acid (= EPA, Figure 21 D), respectively. Figure 21 shows clearly that the reaction products GLA and STA of the Δ6-desaturase OtDes6.1 in the presence of the Δ6-elongase PSE1 are elongated almost quantitatively to give DHGLA and DHSTA, respectively. The subsequent desaturation by the Δ5-desaturase PtD5 to give ARA and EPA, respectively, is also problem-free. Approximately 25-30% of the elongase product is desaturated (Figures 21 B and D).

Table 10 hereinbelow gives an overview of Ostreococcus desaturases which have been cloned:

<u>Ostreococcus tauri desaturases</u>							
Name	bp	aa	Homology	Cyt. B5	His box1	His box2	His box3
OtD4	1611	536	Δ4-desaturase	HPGG	HCANH	WRYHHQVSHH	QVEHHLFP
OtD5.1	606	201	Δ5-desaturase	-	-	-	QVVHHLFP
OtD5.2	714	237	Δ5-desaturase	-	-	WRYHHMVSHH	QIEHHLPF
OtD6.1	1443	480	Δ6-desaturase	HPGG	HEGGH	WNSMHNKHH	QVIHHLFP
OtFAD2	1086	361	Δ12-desaturase	-	HECGH	WQRSHAVHH	HVAHH

Example: 33 Cloning of desaturase genes from Thalassiosira pseudonana

The search for conserved regions in the protein sequences with the aid of conserved motifs (His boxes, see motifs) allowed the identification of six sequences with corresponding motifs in a Thalassiosira pseudonana sequence database (genomic sequences). The sequences are the following:

Name of gene	SEQ ID	Amino acids	Homology
TpD4	SEQ ID NO: 103	503	Δ-4-desaturase
TpD5-1	SEQ ID NO: 99	476	Δ-5-desaturase
TpD5-2	SEQ ID NO: 101	482	Δ-5-desaturase
TpD6	SEQ ID NO: 97	484	Δ-6-desaturase
TpFAD2	SEQ ID NO: 109	434	Δ-12-desaturase
TpO3	SEQ ID NO: 105	418	ω-3-desaturase

Cloning was carried out as follows:

40 ml of a *Thalassiosira pseudonana* culture in the stationary phase were spun down and the pellet was resuspended in 100 µl of double-distilled water and stored at -20°C. The relevant genomic DNAs were amplified based on the PCR method. The corresponding primer pairs were selected in such a way that they contained the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) next to the start codon. The amplification of the TpDes-DNAs was carried out using in each case 1 µl of defrosted cells, 200 µM dNTPs, 2.5 U *Taq* polymerase and 100 pmol of each primer in a total volume of 50 µl. The conditions for the PCR were as follows: first denaturation at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a final elongation step at 72°C for 10 minutes.

Example: 34 Cloning of expression plasmids for the heterologous expression in yeasts:

To characterize the function of the *Thalassiosira pseudonana* desaturases, the open reading frame of the DNA in question is cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), giving rise to the corresponding pYES2.1 clones.

The *Saccharomyces cerevisiae* strain 334 is transformed with the vectors pYES2.1-TpDesaturasen by electroporation (1500 V). A yeast which is transformed with the blank vector pYES2 is used for control purposes. The transformed yeasts are selected on complete minimal medium (CMdum) agar plates supplemented with 2% glucose, but lacking uracil. After the selection, in each case three transformants are chosen for the further functional expression.

To express the Tp desaturases, precultures of in each case 5 ml CMdum liquid medium supplemented with 2% (w/v) raffinose, but lacking uracil, are first inoculated with the transformants chosen and incubated for 2 days at 30°C, 200 rpm. Then, 5 ml of CMdum liquid medium (without uracil) supplemented with 2% of raffinose and 300 µM of various fatty acids are inoculated with the precultures OD_{600} of 0.05. Expression is induced by addition of 2% (w/v) galactose. The cultures are incubated for a further 96 h at 20°C.

Example 35: Cloning of expression plasmids for the seed-specific expression in plants

A further transformation vector based on pSUN-USP is generated for the transformation of plants. To this end, NotI cleavage sites are introduced at the 5' and 3' ends of the coding sequences, using PCR. The corresponding primer sequences are derived from the 5' and 3 regions of the desaturases.

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
5.00 µl 10x buffer (Advantage polymerase)+ 25mM $MgCl_2$
5.00 µl 2mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 min 55°C
Denaturation temperature: 1 min 94°C
Elongation temperature: 2 min 72°C
Number of cycles: 35

The PCR products were incubated with the restriction enzyme NotI for 16 hours at 37°C. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz,P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The

polyadenylation signal is the OCS gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve,H., Dhaese,P., Seurinck,J., Lemmers,M., Van Montagu,M. and Schell,J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982)). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene). (Primer sequence:
5'–GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCC GGATCTGCTGGCTATGAA–3'; SEQ ID NO: 143).
The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

Example 36: Expression of Tp desaturases in yeasts

Yeasts which had been transformed with the plasmids pYES2 and pYES2-Tp desaturases as described in Example 4 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for one hour at 80°C together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programed from 50°C to 250°C with a rate of 5°C/min and finally 10 min at 250°C (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 37: Functional characterization of Thalassiosira pseudonana desaturases:

The substrate specificity of desaturases can be determined after expression in yeast (see Examples cloning of desaturase genes, yeast expression) by feeding, using various yeasts. Descriptions for the determination of the individual activities can be found in WO 93/11245 for Δ15–desaturases, WO 94/11516 for Δ12–desaturases, WO 93/06712, US 5,614,393, US 5614393, WO 96/21022, WO 0021557 and WO 99/27111 for Δ6-desaturases, Qiu et al. 2001, J. Biol. Chem. 276, 31561-31566 for Δ4-desaturases, Hong et al. 2002, Lipids 37,863-868 for Δ5-desaturases.

The activity of the individual desaturases is calculated from the conversion rate using the formula [substrate/(substrate+product)*100].

Tables 11 and 12 which follow give an overview over the cloned Thalassiosira pseudonana desaturases

Table 11: Length and characteristics of the cloned Thalassiosira desaturases.

Desaturase	cDNA (bp)	Protein (aa)	Cyt. B5	His box1	His box2	His box3
TpD4	1512	503	HPGG	HDGNH	WELQHMLGHH	QIEHHLFP
TpD5-1	1431	476	HPGG	HDANH	WMAQHWTHH	QVEHHLFP
TpD5-2	1443	482	HPGG	HDANH	WLAQHWTHH	QVEHHLFP
TpD6	1449	484	HPGG	HDFLH	WKNKHNGHH	QVDHHLFP
TpFAD2 (d12)	1305	434	-	HECGH	HAKHH	HVAHHLFH
TpO3	1257	419	-	HDAGH	WLFMVTYLQH H	HVVHHLF

Table 12: Length, exons, homology and identities of the cloned desaturases.

Des.	GDN A (bp)	Exon 1	Exon 2	First Blast Hit	Hom./Iden.
TpD4	2633	496-1314	1571-2260	Thrautochitrium D4-des	56% / 43%
TpD5-1	2630	490-800	900-2019	Phaeodactylum D5-des	74% / 62%
TpD5-2	2643	532-765	854-2068	Phaeodactylum D5-des	72% / 61%
TpD6	2371	379-480	630-1982	Phaeodactylum D6-des	83% / 69%
TpFAD2	2667	728-2032	-	Phaeodactylum FAD2	76% / 61%
TpO3	2402	403-988	1073-1743	Chaenorhabdidis Fad2	49% / 28%

The Δ 12-desaturase genes from Ostreococcus and Thalassiosira can also be cloned in analogy to the abovementioned examples.

Example 38 Cloning of elongase genes from Xenopus laevis and Ciona intestinalis

By searching for conserved regions (see consensus sequences, SEQ ID NO: 115 and SEQ ID NO: 116) in the protein sequences of the gene databases (Genbank) with the aid of the elongase genes with Δ 5-elongase activity or Δ 6-elongase activity which are detailed in the present application, it was possible to identify and isolate further elongase sequences from other organisms. Using suitable motifs, it was possible to identify further sequences from in each case X. laevis and C. intestinalis, respectively. The sequences were the following:

Name of gene	Organism	Genbank No.	SEQ ID NO:	Amino acids
ELO(XI)	Xenopus laevis	BC044967	117	303
ELO(Ci)	Ciona intestinalis	AK112719	119	290

The X. laevis cDNA clone was obtained from the NIH (National Institute of Health) [Genetic and genomic tools for Xenopus research: The NIH Xenopus initiative, Dev. Dyn. 225 (4), 384-391 (2002)].

The C. intestinalis cDNA clone was obtained from the University of Kyoto [Satou,Y., Yamada,L., Mochizuki,Y., Takatori,N., Kawashima,T., Sasaki,A., Hamaguchi,M., Awazu,S., Yagi,K., Sasakura,Y., Nakayama,A., Ishikawa,H., Inaba,K. and Satoh,N. "A cDNA resource from the basal chordate Ciona intestinalis" JOURNAL Genesis 33 (4), 153-154 (2002)].

Example 39: Cloning of expression plasmids for the heterologous expression in yeasts

The elongase DNAs were amplified with in each case 1 µl cDNA, 200 µM dNTPs, 2,5 U *Advantage* polymerase and 100 pmol of each primer in a total volume of 50 µl. The PCR conditions were as follows: first denaturation at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a last elongation step at 72°C for 10 minutes.

The following oligonucleotides were used for the PCR reaction for cloning the sequence for the heterologous expression in yeasts:

Name of gene, and SEQ ID NO:	Primer sequence
ELO(XI) SEQ ID NO: 121	F:5'- AGGATCCATGGCCTTCAAGGAGCTCACATC
SEQ ID NO: 122	R:5'- CCTCGAGTCAATGGTTTTTGCTTTTCAATGCACCG
ELO(Ci), SEQ ID NO: 123	F:5'- TAAGCTTATGGACGTACTTCATCGT
SEQ ID NO: 124	R:5'- TCAGATCTTTAATCGGTTTTACCATT

*F = forward primer, R = reverse primer

The PCR products were incubated for 30 minutes at 21°C with the yeast expression vector pYES2.1-TOPO (Invitrogen) following the manufacturer's instructions. The PCR product was ligated into the vector by means of a T-overhang and the activity of a topoisomerase (Invitrogen). After the incubation, E. coli DH5α cells are transformed. Suitable clones were identified by PCR, the plasmid DNA was isolated by means of the Qiagen DNAeasy kit and verified by sequencing. The correct sequence was then transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, the blank vector pYES2.1 was transformed in parallel. Thereafter, the yeasts were plated of minimal dropout uracil medium supplemented with 2% glucose. Cells which were capable of growing in the medium without uracil thus comprise the corresponding plasmids pYES2.1, pYES2.1-ELO(XI) und pYES2.1-ELO(Ci). After the selection, in each case two transformants were chosen for the further functional expression.

Example 40: Cloning expression plasmids for the purposes of seed-specific expression in plants

To transform plants, a further transformation vector based on pSUN-USP was generated. To this end, NotI cleavage sites were introduced at the 5' and 3' ends of the coding sequence, using the following primer pair:

pSUN-ELO(XI)

Forward: 5'-GCGGCCGCACCATGGCCTTCAAGGAGCTCACATC (SEQ ID NO: 125)

Reverse: 3'-GCGGCCGCCTTCAATGGTTTTTGCTTTTCAATGCACCG (SEQ ID NO: 126)

pSUN-ELO(Ci)

Forward: 5'-GCGGCCGCACCATGGACGTACTTCATCGT (SEQ ID NO: 127)

Reverse: 3'-GCGGCCGCTTTAATCGGTTTTACCATT (SEQ ID NO: 128)

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
5.00 µl 10 x buffer (Advantage polymerase)+ 25 mM MgCl2
5.00 µl 2 mM dNTP
1.25 µl per primer (10 pmol/µl)
0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 minute at 55°C
Denaturation temperature: 1 minute at 94°C
Elongation temperature: 2 minutes at 72°C
Number of cycles: 35

The PCR products were incubated for 16 hours at 37°C with the restriction enzyme NotI. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the 7624 bp vector were separated by agarose gel electrophoresis and the corresponding ,DNA fragments were excised. The DNA was purified by means of Qiagen gel purification kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation kit from Roche was used for this purpose. The resulting plasmids pSUN-ELO(XI) und

pSUN-ELO(Ci) were verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter as EcoRI fragment into pSUN300. The polyadenylation signal is that of the octopine synthase gene from the A. tumefaciens Ti plasmid (ocs terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982). Der USP promoter corresponds to the nucleotides 1-684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by means of commercially available T7 standard primers (Stratagene) and with the aid of a synthesized primer via a PCR reaction following standard methods

Primer sequence: 5'–
GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCC
GGATCTGCTGGCTATGAA–3' (SEQ ID NO: 129).

The PCR fragment was cut again with EcoRI/SalI and introduced into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

The lipid extraction from yeasts and seeds was as described in Example 6.

Example 41: Expression of ELO(XI) and ELO(Ci) in yeasts

Yeasts which had been transformed with the plasmids pYES2, pYES2-ELO(XI) and pYES2-ELO(Ci) as described in Example 4 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for one hour at 80°C together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C with a rate of 5°C/min and finally 10 min at

250°C (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 42: Functional characterization of ELO(XI) and ELO(Ci):

The substrate specificity of ELO(XI) was determined after expression and after feeding various fatty acids (Fig. 22). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the ELO(XI) reaction. This means that the gene ELO(XI) was expressed functionally.

Table 13 shows that ELO(XI) has a broad substrate specificity. Both C18- and C20-fatty acids are elongated, a preference of Δ5- and Δ6-desaturated fatty acids being observed.

The yeasts which had been transformed with the vector pYES2-ELO(XI) were grown in minimal medium in the presence of the fatty acids stated. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

Table 13: Expression of ELO(XI) in yeast. The conversion rate of various starting materials (fed at in each case 250 μM) is shown.

Starting materials	Conversion of the starting materials by ELO(XI) in %
16:0	3
$16:1^{\Delta 9}$	0
18:0	2
$18:1^{\Delta 9}$	0
$18:2^{\Delta 9,12}$	3
$18:3^{\Delta 6,9,12}$	12
$18:3^{\Delta 5,9,12}$	13

Starting materials	Conversion of the starting materials by ELO(XI) in %
$18:3^{\Delta 9,12,15}$	3
$18:4^{\Delta 6,9,12,15}$	20
$20:3^{\Delta 8,11,14}$	5
$20:3^{\Delta 11,14,17}$	13
$20:4^{\Delta 5,8,11,14}$	15
$20:5^{\Delta 5,8,11,14,17}$	10
$22:4^{\Delta 7,10,13,16}$	0
$22:6^{\Delta 4,7,10,13,16,19}$	0

The substrate specificity of ELO(Ci) was determined after expression and after feeding various fatty acids (Fig. 23). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the ELO(Ci) reaction. This means that the gene ELO(Ci) was expressed functionally

Table 14: Expression of ELO(Ci) in yeast. The conversion rate of various starting materials (fed at in each case 250 µM) is shown.

Starting materials	Conversion of the starting materials by ELO(Ci) in %
16:0	0
$16:1^{\Delta 9}$	0
18:0	0
$18:1^{\Delta 9}$	0
$18:^{2\Delta 9,12}$	23

Starting materials	Conversion of the starting materials by ELO(Ci) in %
$18:3^{\Delta 6,9,12}$	10
$18:3^{\Delta 5,9,12}$	38
$18:3^{\Delta 9,12,15}$	25
$18:4^{\Delta 6,9,12,15}$	3
$20:3^{\Delta 8,11,14}$	10
$20:3^{\Delta 11,14,17}$	8
20:4Δ5,8,11,14	10
20:5Δ5,8,11,14,17	15
22:4Δ7,10,13,16	0
22:6Δ4,7,10,13,16,19	0

Table 14 shows that ELO(Ci) has a broad substrate specificity. Both C18- and C20-fatty acids are elongated, a preference of Δ5- and Δ6-desaturated fatty acids being observed.

The yeasts which had been transformed with the vector pYES2-ELO(Ci) were grown in minimal medium in the presence of the fatty acids stated. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

Example 43: Cloning of genes from Ostreococcus tauri

By searching for conserved regions in the protein sequences with the aid of the elongase genes with Δ5-elongase activity or Δ6-elongase activity, which are described herein, it was possible to identify in each case two sequences with corresponding motifs in an Ostreococcus tauri sequence database (genomic sequences). The sequences were the following:

Name of gene	SEQ ID	Amino acids
OtELO1, (Δ5-elongase)	SEQ ID NO: 67	300
OtELO1.2, (Δ5-elongase)	SEQ ID NO: 113	300
OtELO2, (Δ6-elongase)	SEQ ID NO: 69	292
OtELO2.1, (Δ6-elongase)	SEQ ID NO: 111	292

OtElo1 and OtElo1.2 show the highest degree of similarity to an elongate from Danio rerio (GenBank AAN77156; approx. 26% identity), while OtElo2 and OtElo2.1 show the highest similarity with the Physcomitrella Elo (PSE) [approx. 36% identity] (alignments were carried out using the tBLASTn algorithm (Altschul et al., J. Mol. Biol. 1990, 215: 403 – 410).

The elongases were cloned as follows:

40 ml of an *Ostreococcus tauri* culture in the stationary phase were spun down, resuspended in 100 μl of double-distilled water and stored at -20°C. Based on the PCR method, the respective genomic DNAs were amplified. The respective primer pairs were chosen in such a way that they bore the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) adjacent to the start codon. The OtElo DNAs were amplified in each case using 1 μl of defrosted cells, 200 μM dNTPs, 2.5 U *Taq* polymerase and 100 pmol of each primer in a total volume of 50 μl. The PCR conditions were as follows: first denaturation at 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a last elongation step at 72°C for 10 minutes.

Example 44: Cloning of expression plasmids for the heterologous expression yeasts:

To characterize the function of the *Ostreococcus tauri* elongases, the open reading frame of the DNA in question is cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), giving rise to the corresponding pOTE1, pOTE1.2, pOTE2 and pOTE2.1 clones.

The *Saccharomyces cerevisiae* strain 334 is transformed with the vectors pOTE1, pOTE1.2, pOT22 and pOTE2.1, respectively by electroporation (1500 V). A yeast which is transformed with the blank vector pYES2 is used for control purposes. The transformed yeasts are selected on complete minimal medium (CMdum) agar plates supplemented with 2% glucose, but lacking uracil. After the selection, in each case three transformants are chosen for the further functional expression.

To express the Ot elongases, precultures of in each case 5 ml CMdum liquid medium supplemented with 2% (w/v) raffinose, but lacking uracil, are first inoculated with the transformants chosen and incubated for 2 days at 30°C, 200 rpm.
Then, 5 ml of CMdum liquid medium (without uracil) supplemented with 2% of raffinose

and 300 µM of various fatty acids are inoculated with the precultures OD_{600} of 0.05. Expression is induced by addition of 2% (w/v) galactose. The cultures are incubated for a further 96 h at 20°C.

Example 45: Cloning of expression plasmids for the seed-specific expression in plants

A further transformation vector based on pSUN-USP is generated for the transformation of plants. To this end, NotI cleavage sites are introduced at the 5' and 3' ends of the coding sequences, using PCR. The corresponding primer sequences are derived from the 5' and 3 regions of OtElo1, OtElo1.2, OtElo2 and OtElo2.1.

Composition of the PCR mix (50 µl):

5.00 µl template cDNA
5.00 µl 10x buffer (Advantage polymerase)+ 25mM $MgCl_2$
5.00 µl 2mM dNTP
1.25 µl of each primer (10 pmol/µl)
0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed.
PCR reaction conditions:
Annealing temperature: 1 min 55°C
Denaturation temperature: 1 min 94°C
Elongation temperature: 2 min 72°C
Number of cycles: 35

The PCR products were incubated with the restriction enzyme NotI for 16 hours at 37°C. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the vector were separated by agarose gel electrophoresis and the corresponding DNA fragments were excised. The DNA was purified by means of the Qiagen Gel Purification Kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation Kit from Roche was used for this purpose. The resulting plasmids were verified by pSUN-OtEllO1, pSUN-OtELO1.2, pSUN-OtELO2 and pSUN-OtELO2.2 sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz,P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter into pSUN300 in the form of an EcoRI fragment. The polyadenylation signal is that of the Ostreococcus gene from the A. tumefaciens Ti plasmid (ocs-Terminator, Genbank Accession V00088) (De Greve,H., Dhaese,P., Seurinck,J., Lemmers,M., Van Montagu,M. and Schell,J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982). The USP promoter corresponds to nucleotides 1 to 684 (Genbank Accession X56240), where part of the

noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by a PCR reaction and standard methods with the aid of a synthesized primer and by means of a commercially available T7 standard primer (Stratagene).

(Primer sequence:

5'–GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCC GGATCTGCTGGCTATGAA–3'). (SEQ ID NO: 130)

The PCR fragment was recut with EcoRI/SalI and inserted into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

Example 46: Expression of OtElo1, OtElo1.2, OtElo2 and OtELO2.2 in yeasts

Yeasts which had been transformed with the plasmids pYES3, pYES3-OtELO1, pYES3-OtELO1.2, pYES3-OtELO2 and pYES3-OtELO2.2 as described in Example 15 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for one hour at 80°C together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programed from 50°C to 250°C with a rate of 5°C/min and finally 10 min at 250°C (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 47: Functional characterization of OtElo1, OtElo1.2, OtElo2 and OtElo2.1:

The substrate specificity of OtElo1 was determined after expression and after feeding various fatty acids (Tab. 15). The substrates fed can be detected in large amounts in all

of the transgenic yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the OtElo1 reaction. This means that the gene OtElo1 was expressed functionally.

Table 15 shows that OtElo1 and OtElo1.2 have a narrow substrate specificity. OtElo1 and OtElo1.2 were only capable of elongating the C20-fatty acids eicosapentaenoic acid (Figure 24A, 24B) and arachidonic acid (Figure 25A, 25B), but preferred eicosapentaenoic acid, which is ω-3-desaturated.

Table 15 shows the substrate specificity of the elongase OtElo1 and OtElo1.2 for C20-polyunsaturated fatty acids with a double bond in the Δ5 position in comparison with various fatty acids.

The yeasts which had been transformed with the vector pOTE1 and pOTE1.2, respectively, were grown in minimal medium in the presence of the fatty acids stated. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

The substrate specificity of OtElo1 (SEQ ID NO: 81) OtElo2.1 (SEQ ID NO: 111) was determined after expression and after feeding various fatty acids (Tab. 16). The substrates fed can be detected in large amounts in all of the transgenic yeasts. The transgenic yeasts revealed the synthesis of novel fatty acids, the products of the OtElo2 reaction. This means that the genes OtElo2 and OtElo2.1 was expressed functionally.

Table 15:

Fatty acid substrate	**Conversion rate (in %) OtElo1**	**Conversion rate (in %) OtElo1.2**
16:0	-	-
$16:1^{\Delta 9}$	-	-
18:0	-	-
$18:1^{\Delta 9}$	-	-
$18:1^{\Delta 11}$	-	-
$18:2^{\Delta 9,12}$	-	-
$18:3^{\Delta 6,9,12}$	-	-
$18:3^{\Delta 5,9,12}$	-	-

Fatty acid substrate	Conversion rate (in %) OtElo1	Conversion rate (in %) OtElo1.2
20:3 $^{\Delta8,11,14}$	-	-
20:4 $^{\Delta5,8,11,14}$	**10.8 ± 0.6**	**38.0**
20:5 $^{\Delta5,8,11,14,17}$	**46.8 ± 3.6**	**68.6**
22:4 $^{\Delta7,10,13,16}$	-	-
22:6$^{\Delta4,7,10,13,16,19}$	-	-

Table 16 shows the substrate specificity of the elongase OtElo2 and OtElo2.1 for various fatty acids. The activity of OtElo2.1 is markedly higher.

The yeasts which had been transformed with the vector pOTE2 and pOTE2.1, respectively, were grown in minimal medium in the presence of the fatty acids stated. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC.

The enzymatic activity which is shown in Table 16 demonstrates clearly that OtElo2, or OtElo2.1, is a Δ6-elongase.

Table 16:

Fatty acid substrate	Conversion rate (in %) OtElo2	Conversion rate (in %) OtELO2.2
16:0	-	-
16:1$^{\Delta9}$	-	-
16:3$^{\Delta7,10,13}$	-	-
18:0	-	-
18:1$^{\Delta6}$	-	-
18:1$^{\Delta9}$	-	-
18:1$^{\Delta11}$	-	-
18:2$^{\Delta9,12}$	-	-
18:3$^{\Delta6,9,12}$	**15.3**	**55.7**
18:3 $^{\Delta5,9,12}$	-	-
18:4 $^{\Delta6,9,12,15}$	**21.1**	**70.4**
20:2 $^{\Delta11,14}$	-	-

20:3 $^{\Delta 8,11,14}$	-	-
20:4 $^{\Delta 5,8,11,14}$	-	-
20:5 $^{\Delta 5,8,11,14,17}$	-	-
22:4 $^{\Delta 7,10,13,16}$	-	-
22:5 $^{\Delta 7,10,13,16,19}$	-	-
22:6$^{\Delta 4,7,10,13,16,19}$	-	-

Figure 24 A – D shows the elongation of eicosapentaenoic acid by OtElo1 (B) and OtElo1.2 (D), respectively. The controls (A, C) do not show the elongation product (22:5ω3).

Figure 25 A – D shows the elongation of arachidonic acid by OtElo1 (B) and OtElo1.2 (D), respectively. The controls (A, C) do not show the elongation product (22:4ω6).

Example 48: Cloning of elongase genes from Euglena gracilis and Arabidopsis thaliana

By searching for conserved regions in the protein sequences with the aid of the elongase genes with Δ5-elongase activity or Δ6-elongase activity, which are detailed in the application, it was possible to identify sequences from Arabidopsis thaliana and Euglena gracilis, respectively, with corresponding motifs in sequence databases (Genbank, Euglena EST library). The sequences are the following:

Name of gene	SEQ ID	Amino acids
EGY1019 (E. gracilis)	SEQ ID NO: 131	262
EGY2019 (E. gracilis)	SEQ ID NO: 133	262
At3g06460 (A. thaliana)	SEQ ID NO: 135	298
At3g06470 (A. thaliana)	SEQ ID NO: 137	278

The Euglena gracilis elongases were cloned as follows:

The Euglena gracilis strain 1224-5/25 was obtained from the Sammlung für Algenkulturen Göttingen [Göttingen collection of algal cultures] (SAG). For the isolation, the strain was grown in medium II (Calvayrac R and Douce R, FEBS Letters 7:259-262, 1970) for 4 days at 23°C with a light/dark interval of 8 h/16 h (light intensity 35 mol s-1 m-2).

Total RNA of a four-day-old Euglena culture was isolated with the aid of the RNAeasy kit from Qiagen (Valencia, CA, US). Poly-A+ RNA (mRNA) was isolated from the total RNA with the aid of oligo-dt-cellulose (Sambrook et al., 1989). The RNA was subjected to reverse transcription using the Reverse Transcription System kit from Promega, and the cDNA synthesized was cloned into the vector lambda ZAP (lambda ZAP Gold, Stratagene). The cDNA was depackaged in accordance with the manufacturer's

instructions to give plasmid DNA, and clones were part-sequenced for random sequencing. mRNA was isolated from the total RNA with the aid of the PolyATract isolation system (Promega). The mRNA was subjected to reverse transcription using the Marathon cDNA amplification kit (BD Biosciences), and the adapters were ligated in accordance with the manufacturer's instructions. The cDNA library was then used for the PCR for cloning expression plasmids by means of 5' and 3'-RACE (rapid amplification of cDNA ends).

The Arabidopsis thaliana elongases were cloned as follows:

Starting from the genomic DNA, primers for the two genes were derived in each case at the 5' and 3' end of the open reading frame.

The method of Chrigwin *et al.*, (1979) was used for isolating total RNA from *A. Thaliana*. Leaves of 21-day-old plants were comminuted with a pestle and mortar in liquid nitrogen, treated with disruption buffer and incubated for 15 minutes at 37°C. After centrifugation (10 min, 4°C, 12 000 × g), the RNA in the supernatant was precipitated with 0.02 volume of 3 M sodium acetate pH 5.0 and 0.75 volume of ethanol at -20°C for 5 hours. Then, after a further centrifugation step, the RNA was taken up in 1 ml of TES per g of starting material, extracted once with one volume of phenol/chloroform and once with one volume of chloroform, and the RNA was precipitated with 2.5 M LiCl. After the subsequent centrifugation and washing with 80% ethanol, the RNA was resuspended in water. The cDNA was synthesized as described by Sambrook et al. 1989, and RT-PCR was carried out with the derived primers. The PCR products were cloned into the vector pYES2.1-TOPO (Invitrogen) following the manufacturer's instructions.

Example 49: Cloning of expression plasmids for the heterologous expression in yeasts:

To characterize the function of the *A. thaliana* desaturases, the open reading frame of the DNA in question is cloned downstream of the galactose-inducible GAL1 promoter of pYES2.1/V5-His-TOPO (Invitrogen), giving rise to the corresponding pAt60 and pAt70 clones.

The *Saccharomyces cerevisiae* strain 334 is transformed with the vectors pAt60 and pAt70, respectively by electroporation (1500 V). A yeast which is transformed with the blank vector pYES2.1 is used for control purposes. The transformed yeasts are selected on complete minimal medium (CMdum) agar plates supplemented with 2% glucose, but lacking uracil. After the selection, in each case three transformants are chosen for the further functional expression.

To express the At elongases, precultures of in each case 5 ml of CMdum liquid medium supplemented with 2% (w/v) raffinose, but without uracil, were inoculated with

the selected transformants and incubated for 2 hours at 30°C, 200 rpm.

5 ml of CMdum liquid medium (without uracil) supplemented with 2% of raffinose and 300 μM various fatty acids were then inoculated with the precultures to an OD_{600} of 0.05. Expression was induced by the addition of 2% (w/v) galactose. The cultures were incubated for a further 96 hours at 20°C.

Example 50: Expression of pAt60 and pAt70 in yeasts

Yeasts which had been transformed with the plasmids pYES2.1, pAt60 and pAt70, respectively, as described in Example 5 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100 x g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs) were prepared by acid methanolysis. To this end, the cell sediments were incubated for one hour at 80°C together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 μl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 μm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C with a rate of 5°C/min and finally 10 min at 250°C (holding).

The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 51: Functional characterization of pAt60 and pAt70

The substrate specificity of the elongases At3g06460 and At3g06470, respectively, was determined followed expression and feeding of various fatty acids (Tab. 17, Fig. 26). The substrates fed can be detected in all of the transgenic yeasts, The transgenic yeasts showed the synthesis of new fatty acids, the products of the genes At3g06460 and At3g06470, respectively. This means that these genes were expressed functionally.

Table 17: Elongation of EPA by the elongasen At3g06460 and At3g06470, respectively. Analysis of the yeast extracts after feeding with 250 uM EPA.

Gene	Fatty acid fed	C20:5n-3 content	C22:5n-3 content
At3g06460	EPA (C20:5n-3)	20.8	0.6
At3g06460	EPA (C20:5n-3)	25.4	1.1
Conversion rate of EPA	At3g06460: 3.0%	At3g06470: 4.1%	

Figure 26 shows the elongation of 20:5n-3 by the elongases At3g06470.

Example 52: Cloning of an elongase from Phaeodactylum tricornutum

Starting from conserved regions in the protein sequences with the aid of the elongase genes with Δ6-elongase activity detailed in the application, degenerate primers were generated and these primers were used for screening a *Phaeodactylum* cDNA library by means of PCR. The following primer sequences were employed:

Name of primer	Sequence 5'-3' orientation	Corresponding amino acids
Phaelo forward1	AA(C/T)CTUCTUTGGCTUTT(C/T)TA (SEQ ID NO. 185)	NLLWLFY
Phaelo reverse1	GA(C/T)TGUAC(A/G)AA(A/G)AA(C/T)TGUG C(A/G)AA (SEQ ID NO. 186)	FAQFFVQS

Nucleotide bases in brackets mean that a mixture of oligonucleotides with in each case one or the other nucleotide base is present.

Preparation of the *Phaeodactylum* cDNA library:

A 2 l culture of P. tricornutum UTEX 646 was grown in f/2 medium (Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. In *Culture of Marine Invertebrate Animals* (Eds. Smith, W.L. and Chanley, M.H.), Plenum Press, New York, pp 29–60) for 14 days at a light intensity of 35 E/cm^2. After centrifugation, frozen cells were ground to a fine powder in the presence of liquid nitrogen and resuspended in 2 ml of homogenization buffer (0.33 M sorbitol 0.3 M, NaCl, 10 mM EDTA, 10 mM EGTA, 2% SDS, 2% mercaptoethanol in 0.2 M Tris-Cl pH 8.5). After addition of 4 ml of phenol and 2 ml of chloroform, the mixture was shaken vigorously for 15 minutes at 45-50°C. It was subsequently centrifuged (10 min × 10 000 g), and the aqueous phase was extracted stepwise using chloroform. Nucleic acids were then precipitated by addition of 1/20 volume of 4 M sodium hydrogencarbonate solution and centrifuged. The pellet was taken up in 80 mM Tris-borate pH 7.0 and 1 mM EDTA, and the RNA

was precipitated with 8 M lithium chloride. After centrifugation and washing with 70% ethanol, the RNA pellet was taken up in RNase-free water. Poly(A)-RNA was isolated with Dynabeads (Dynal, Oslo, Norway) following the manufacturer's instructions, and the first-strain cDNA synthesis was carried out using MLV-Rtase from Roche (Mannheim). The second-strand synthesis was then carried out by means of DNA polymerase I and Klenow fragment, followed by RNase H digestion. The cDNA was treated with T4 DNA polymerase, and EcoRI/XhoI adaptors (Pharmacia, Freiburg) were subsequently attached by means of T4 ligase. After XhoI digestion, phosphorylation and gel separation, fragments greater than 300 bp were ligated into the phage lambda ZAP Express following the manufacturer's instructions (Stratagene, Amsterdam, The Netherlands). After mass excision of the cDNA library and plasmid recovery, the plasmid library was transformed into E. coli DH10B cells and employed for PCR screening.

Using the abovementioned degenerate primers, it was possible to generate the PCR fragment with the SEQ ID NO: 187.

This fragment was labeled with digoxigenin (Roche, Mannheim) and used as probe for screening the phage library.

Using the sequence SEQ ID NO: 187, it was possible to obtain the gene sequence SEQ ID NO: 183, which constitutes the full-length RNA molecule of the Phaeodactylum Δ6-elongase:

Example 53: Cloning of expression plasmids for the heterologous expression in yeasts

The primer pairs in question were chosen in such a way that they bore the yeast consensus sequence for highly efficient translation (Kozak, Cell 1986, 44:283-292) next to the start codon. The PtELO6 DNA was amplified with in each case 1 µl of cDNA, 200 µM dNTPs, 2.5 U of Advantage polymerase and 100 pmol of each primer in a total volume of 50 µl. The PCR conditions were as follows: first denaturation of 95°C for 5 minutes, followed by 30 cycles at 94°C for 30 seconds, 55°C for 1 minute and 72°C for 2 minutes, and a last elongation step at 72°C for 10 minutes.

The following oligonucleotides were used for the PCR reaction for cloning the sequence for the heterologous expression in yeasts:

Name of gene, and SEQ ID NO:	Primer sequence
PtELO6 (SEQ ID NO: 183)	F:5'-GCGGCCGCACATAATGATGGTACCTTCAAG (SEQ ID NO: 188) R:3'- GAAGACAGCTTAATAGACTAGT

	(SEQ ID NO: 189)

*F=forward primer, R=reverse primer

The PCR products were incubated for 30 minutes at 21°C with the yeast expression vector pYES2.1-TOPO (Invitrogen) following the manufacturer's instructions. The PCR product (see SEQ ID NO: 192) was ligated into the vector by means of a T-overhang and the activity of a topoisomerase (Invitrogen). After the incubation, E. coli DH5α cells were transformed. Suitable clones were identified by PCR, the plasmid DNA was isolated by means of the Qiagen DNAeasy kit and verified by sequencing. The correct sequence was then transformed into the Saccharomyces strain INVSc1 (Invitrogen) by electroporation (1500 V). As a control, the blank vector pYES2.1 was transformed in parallel. Thereafter, the yeasts were plated of minimal dropout uracil medium supplemented with 2% glucose. Cells which were capable of growing in the medium without uracil thus comprise the corresponding plasmids pYES2.1 and pYES2.1-PtELO6. After the selection, in each case two transformants were chosen for the further functional expression.

Example 54: Cloning expression plasmids for the purposes of seed-specific expression in plants

To transform plants, a further transformation vector based on pSUN-USP was generated. To this end, NotI cleavage sites were introduced at the 5' and 3' ends of the coding sequence, using the following primer pair:

PSUN-PtELO6

Forward: 5'-GCGGCCGCACCATGATGGTACCTTCAAGTTA (SEQ ID NO: 190)
Reverse: 3'-GAAGACAGCTTAATAGGCGGCCGC (SEQ ID NO: 191)

Composition of the PCR mix (50 µl):
5.00 µl template cDNA
5.00 µl 10 x buffer (Advantage polymerase)+ 25 mM MgCl2
5.00 µl 2 mM dNTP
1.25 µl per primer (10 pmol/µl)
0.50 µl Advantage polymerase

The Advantage polymerase from Clontech was employed.

PCR reaction conditions:

Annealing temperature: 1 minute at 55°C
Denaturation temperature: 1 minute at 94°C
Elongation temperature: 2 minutes at 72°C

Number of cycles: 35

The PCR products were incubated for 16 hours at 37°C with the restriction enzyme NotI. The plant expression vector pSUN300-USP was incubated in the same manner. Thereafter, the PCR products and the 7624 bp vector were separated by agarose gel electrophoresis and the corresponding ,DNA fragments were excised. The DNA was purified by means of Qiagen gel purification kit following the manufacturer's instructions. Thereafter, vector and PCR products were ligated. The Rapid Ligation kit from Roche was used for this purpose. The resulting plasmid pSUN-PtELO was verified by sequencing.

pSUN300 is a derivative of plasmid pPZP (Hajdukiewicz, P, Svab, Z, Maliga, P., (1994) The small versatile pPZP family of Agrobacterium binary vectors for plant transformation. Plant Mol Biol 25:989-994). pSUN-USP originated from pSUN300, by inserting a USP promoter as EcoRI fragment into pSUN300. The polyadenylation signal is that of the octopine synthase gene from the A. tumefaciens Ti plasmid (ocs terminator, Genbank Accession V00088) (De Greve, H., Dhaese, P., Seurinck, J., Lemmers, M., Van Montagu, M. and Schell, J. Nucleotide sequence and transcript map of the Agrobacterium tumefaciens Ti plasmid-encoded octopine synthase gene J. Mol. Appl. Genet. 1 (6), 499-511 (1982). Der USP promoter corresponds to the nucleotides 1-684 (Genbank Accession X56240), where part of the noncoding region of the USP gene is present in the promoter. The promoter fragment which is 684 base pairs in size was amplified by means of commercially available T7 standard primers (Stratagene) and with the aid of a synthesized primer via a PCR reaction following standard methods.

(Primer sequence: 5'–
GTCGACCCGCGGACTAGTGGGCCCTCTAGACCCGGGGGATCC
GGATCTGCTGGCTATGAA–3'; SEQ ID NO: 151).

The PCR fragment was cut again with EcoRI/SalI and introduced into the vector pSUN300 with OCS terminator. This gave rise to the plasmid with the name pSUN-USP. The construct was used for the transformation of Arabidopsis thaliana, oilseed rape, tobacco and linseed.

The lipid extraction from yeasts and seeds was as described in Example 6.

Example 55: Expression of PtElo in yeasts

Yeasts which had been transformed with the plasmids pYES2 and pYES2-PtELO6 as described in Example 4 were analyzed as follows:

The yeast cells from the main cultures were harvested by centrifugation (100 × g, 5 min, 20°C) and washed with 100 mM $NaHCO_3$, pH 8.0 to remove residual medium and fatty acids. Starting with the yeast cell sediments, fatty acid methyl esters (FAMEs)

were prepared by acid methanolysis. To this end, the cell sediments were incubated for one hour at 80°C together with 2 ml of 1 N methanolic sulfuric acid and 2% (v/v) of dimethoxypropane. The FAMEs were extracted twice with petroleum ether (PE). To remove nonderivatized fatty acids, the organic phases were washed in each case once with 2 ml of 100 mM $NaHCO_3$, pH 8.0 and 2 ml of distilled water. Thereafter, the PE phases were dried with Na_2SO_4, evaporated under argon and taken up in 100 µl of PE. The samples were separated on a DB-23 capillary column (30 m, 0.25 mm, 0.25 µm, Agilent) in a Hewlett-Packard 6850 gas chromatograph equipped with flame ionization detector. The conditions for the GLC analysis were as follows: the oven temperature was programmed from 50°C to 250°C with a rate of 5°C/min and finally 10 min at 250°C (holding).
The signals were identified by comparing the retention times with corresponding fatty acid standards (Sigma). The methodology is described for example in Napier and Michaelson, 2001, Lipids. 36(8):761-766; Sayanova et al., 2001, Journal of Experimental Botany. 52(360):1581-1585, Sperling et al., 2001, Arch. Biochem. Biophys. 388(2):293-298 and Michaelson et al., 1998, FEBS Letters. 439(3):215-218.

Example 56: Functional characterization of PtELO6:

Figure 29 shows the conversion of $C18:3^{\Delta6,9,12}$ and $C18:4^{\Delta6,9,12,15}$. The substrates are elongated by in each case two carbon atoms; the fatty acids $C20:3^{\Delta8,11,14}$ and $C20:4^{\Delta8,11,14,17}$ are formed, respectively. The substrate specificity of PtELO6 was determined after expression and feeding various fatty acids (Fig. 30). Large amounts of the substrates fed can be detected in all of the transgenic yeasts. The transgenic yeasts show the synthesis of new fatty acids, the products of the PtElo6 reaction. This means that the gene PtELO6 has been expressed functionally.

Table 18 shows that PtElo6 has a narrow substrate specificity. PtELO6 was only able to elongate the C18-fatty acids linoleic acid, linolenic acid, γ-linolenic acid and stearidonic acid, but preferred stearidonic acid, which is ω3-desaturated (see also Figure 30).

Feeding experiment: fatty acids (in bold) were added at in each case 250 µm. The formation of the underlying fatty acids is new.

Table 18: Substrate specificity of PtElo6

Fatty acid fed:		**+ 18:2**	**+ 18:3**	**+ 18:3**	**+ 18:4**
16:0	16.2	18.2	15.2	20	04:48
16:1	50.6	20.5	22.8	33.5	34.2
18:0	5.4	6.3	6.2	5.2	12.4
18:1	27.7	14.6	19.6	19.3	16.7
18:2		40			
18:3			32.9		
18:3				12.3	
18:4					4.5
20:2		0.4			

20:3			3.4		
20:3				9.7	
20:4					14.5
% Elongation	**0.0**	**0.99**	**9.37**	**44.09**	**76.32**

The following fatty acids were fed, but not converted:

- $18:1^{\Delta 6}$, $18:1^{\Delta 9}$, $18:1^{\Delta 11}$
- $20:2^{\Delta 11,14}$, $20:3^{\Delta 11,14,17}$, $20:3^{\Delta 8,11,14}$, $20:4^{\Delta 5,8,11,14}$, $20:5^{\Delta 5,8,11,14,17}$
- $22:4^{\Delta 7,10,13,16}$

The yeasts which had been transformed with the vector pYES2-PtELO6 were grown in minimal medium in the presence of the fatty acids stated. The fatty acid methyl esters were synthesized by subjecting intact cells to acid methanolysis. Thereafter, the FAMEs were analyzed via GLC. In this way, the results which were shown in Figures 29 and 30 and in Table 16 were obtained.

Equivalents:

Many equivalents of the specific embodiments according to the invention described herein can be identified or found by the skilled worker resorting simply to routine experiments. These equivalents are intended to be within the scope of the patent claims.

Table 3: Conversion rates of the fatty acids fed. The conversion rates were calculated using the formula: [conversion rate]=[product]/[substrate]+[product]*100.

BioTaur clones area in % of the GC analysis														
Clone	Fatty acid	C16:0	C16:1 (n-7)	C18:0	C18:1 (n-9)	C18:3 (n-6)	C18:4 (n-3)	C20:3 (n-6)	C20:4 (n-6)	C20:4 (n-3)	C20:5 (n-3)	C22:4 (n-6)	C22:4 (n-3)	C22:5 (n-3)
Vector	none	21.261	41.576	4.670	25.330									
BioTaur	none	20.831	37.374	4.215	26.475									
Vector	GLA + EPA	22.053	23.632	5.487	17.289	**11.574**					**13.792**			
BioTaur	GLA + EPA	20.439	25.554	6.129	19.587	**3.521**		**6.620**			**10.149**			**1.127**
Vector	EPA	20.669	28.985	6.292	21.712						**16.225**			
BioTaur	EPA	20.472	26.913	6.570	23.131						**11.519**			**3.251**
Vector	ARA	23.169	23.332	6.587	12.735				**27.069**					
BioTaur	ARA	20.969	31.281	5.367	21.351				**9.648**			**1.632**		
Vector	SDA	18.519	12.626	6.642	6.344		**47.911**							
BioTaur	SDA	19.683	15.878	7.246	8.403		**13.569**			**25.946**			**0.876**	